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# CEREAL CHEMISTRY

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## BIOCHEMISTRY OF THE DAMAGE TO GRAIN BY THE WHEAT-BUG

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One of the most serious types of wheat damage in Europe, Asia, and North Africa is that caused by insects of such species as Eurygaster, Aelia, and Dolycoris, first described by Berliner (1931). These insects, generally called wheat-bugs, attack the kernel during the ripening period and lower its viability and baking quality. The data obtained by Kretovich, Bundel, and Pschenova (1943), reproduced in Table I, illustrate the reduction in viability.

TABLE I  
VIABILITY OF WHEAT INFECTED BY THE WHEAT-BUG

Sample	Infected kernels	Viability		
		Entire sample	Normal kernels	Damaged kernels
	%	%	%	%
1	3.8	94.8	95.0	89.1
2	8.6	92.1	96.0	50.9
3	13.1	90.7	98.0	41.8
4	14.3	85.8	95.0	30.9
5	19.2	79.1	90.0	32.7
6	24.3	67.9	82.0	24.0

The deterioration in baking quality is manifested by the fact that the flour from highly damaged grain either does not form gluten or the gluten can hardly be washed out; it sticks to the fingers, has no elasticity, and upon standing, disintegrates into a sticky mass. Consequently, dough prepared from damaged flour also disintegrates, is hard to mold, and produces flat loaves of poor crumb texture.

Investigations have shown that wheat-bugs puncture the ripening kernel and suck out a portion of the contents. Consequently the

kernel, though ripening and reaching full maturity, is of inferior viability and baking quality.

After harvest, a wheat kernel which has been punctured by the wheat-bug is shrivelled, has lower absolute weight, and shows a pale area with or without a small dark spot on its surface. The pale area is usually slightly shrivelled and is limited to the damaged region. The endosperm in the damaged region is very soft and can be easily crumbled out, whereas in the undamaged part of the kernel it is normally hard. If the kernel has been pierced many times by the wheat-bug it contains almost no endosperm.

Upon examining a cross section through the pierced area of a damaged kernel under the microscope, one may see that a considerable part of the protein in that area has been dissolved (Fleckinger, 1936). It is natural to suppose that the bug, after having punctured the kernel, injects secretions which cause the dissolution of the contents of the endosperm cells. Actually, Hibraoui (1930) and Malenotti (1933) have observed that wheat-bugs inject a liquid when piercing the kernel.

The experiments reported here were designed to determine the biochemical properties of grain damaged by wheat-bugs.

### Experimental

In studying the effect of the wheat-bug on the protein complex of the kernel the first consideration is to examine the total nitrogen in the damaged kernel. The analysis of normal and infected kernels, selected by hand, showed that the total nitrogen content is decreased as a result of the damage caused by the wheat-bug (Table II).

TABLE II  
TOTAL NITROGEN CONTENT OF NORMAL AND INFECTED KERNELS

Sample	Nitrogen <sup>1</sup>	
	Normal	Infected
	%	%
1	2.03	1.88
2	2.28	2.02
3	2.43	2.14
4	2.24	2.07
5	2.26	2.10
6	2.17	1.95

<sup>1</sup> Dry matter basis.

These results indicate that wheat-bugs, in piercing the kernel, extract part of the nitrogenous substances.

More detailed analyses of normal and infected kernels are given in Table III.

These data show that the titratable acidity of flour from damaged grain is higher than that for normal grain. The amylase activity is also higher in the flour from infected grain, indicating that the wheat-bug introduces amylase into the kernel.

TABLE III  
BIOCHEMICAL ANALYSES OF NORMAL AND INFECTED KERNELS

Characteristics of kernels	Nitrogen				Nitrogen of gliadin as percent of total gluten nitrogen	pH of flour suspen- sion	Titratable acidity of extract		Activity of	
	Total	Water- soluble, as per- cent of total	Gli- adin nitro- gen as per- cent of total	In glu- ten			Water	Alco- hol	Cata- lase <sup>1</sup>	Amyl- ase <sup>2</sup>
Normal kernels	2.70	14.54	47.2	15.3	51.6	6.6	2.3	3.0	38.9	114.4
Damaged kernels (with pale areas and dark spots)	2.59	28.0	54.2	14.6	71.8	6.5	2.7	4.2	37.8	163.6
Damaged kernels (without dark spots)	2.51	29.8	55.1	—	—	—	—	—	—	—

<sup>1</sup> Method of Bach and Oparin (1923).

<sup>2</sup> Method of Rumsey (1922).

The nitrogen fractionation studies show that infection by the wheat-bug considerably increases the solubility of the proteins in both water and 60% alcohol. It is thus clear that the nitrogenous substances of the damaged kernels in particular underwent profound changes; the gluten had about 75% of gliadin nitrogen instead of the usual 50%.

Since the high water-soluble nitrogen values for the infected kernels suggested extensive proteolysis, it seemed necessary to examine the relative changes in nitrogen solubility which occurred when normal and infected wheats were subjected to autolysis. As a measure of proteolytic activity, the total water-soluble nitrogen and the nitrogen not precipitated by 2% trichloroacetic acid solution were determined before and after autolysis. The results given in Table IV demonstrate the considerable increase (during autolysis) of nitrogen not precipitated by trichloroacetic acid. This is characteristic of peptones, peptides, and amino acids formed as a result of extensive enzymatic splitting of proteins in the damaged grain. In line with these data, Geoffroy (1936) reported a greater increase in amino nitrogen during the autolysis of damaged grain as compared with normal grain.

To determine more definitely the changes which the protein substances undergo when subjected to the effect of wheat-bugs, pure

gliadin preparations were made from normal and infected kernels by Kretovich's (1937) acetone method. The very process of obtaining the preparations confirmed the greater solubility of the proteins from infected kernels in comparison with the controls: a yield of only 2.6 g

TABLE IV  
CHANGE IN SOLUBLE NITROGEN FRACTIONS OF NORMAL AND DAMAGED KERNELS DURING AUTOLYSIS

Sample No.	Kernels	Nitrogen in percent of total nitrogen			
		Before autolysis		After autolysis	
		Water-soluble	Not precipitated by $\text{CCl}_3\text{COOH}$	Water-soluble	Not precipitated by $\text{CCl}_3\text{COOH}$
1	Normal	% 12.5	% 7.6	% 22.7	% 16.1
	Damaged	22.0	16.3	52.7	43.8
2	Normal	10.0	4.7	21.2	13.4
	Damaged	19.9	14.0	56.9	42.3

of gliadin was secured from 250 g of infected kernels, whereas from 250 g of normal kernels, under the same conditions, 6.7 g was obtained. The sharp reduction in gliadin yield from infected kernels may be explained by its dispersion in water during washing.

Investigation of the physical and chemical properties of the gliadins showed (Table V) that the protein of the infected kernels under-

TABLE V  
PROPERTIES OF GLIADIN FROM NORMAL AND INFECTED KERNELS

Source of gliadin	Nitrogen	Sulfur	Tryptophane (Furth-Diesche)	Viscosity <sup>1</sup>	$[\alpha]_D^{20}$ in 60% ethanol
Normal kernels	% 17.6	% 0.97	% 0.91	3.008	-91.8
Infected kernels	17.1	1.37	0.87	2.737	-72.5

<sup>1</sup> Viscosity values are given as the ratio of the viscosity of 1% gliadin solution in 60% alcohol to the viscosity of water.

goes deep intramolecular changes which result in a considerable lowering of the viscosity and specific rotation of gliadin solutions as well as in a marked increase in the sulfur content of the gliadin. The decrease in viscosity and the change in optical activity of protein solutions during autolysis are recognized phenomena which may serve as quantitative measures of proteolysis. It is possible, of course, that the pronounced differences in the optical properties of the gliadin prepared

from normal and infected kernels may be caused by the presence of glutenin cleavage products in the gliadin prepared from the damaged kernels.

The high sulfur content of the gliadin from the infected kernels is of particular interest. Evidently the proteolysis caused by the secretions of the wheat-bug does not involve the liberation of sulfur-containing amino acids, such as cystine and methionine, from the protein molecule. A similar observation was made by Chittenden and Smith (1890) who found that the colloidal products of the hydrolysis of wheat glutenin by pepsin contained much more sulfur than the original protein.

The question arises whether the secretions of the bug are concentrated in the region of the puncture or whether they extend throughout

TABLE VI  
NITROGEN FRACTIONS OF NORMAL AND DAMAGED PARTS OF INFECTED  
WHEAT KERNELS

Sample No.	Part of kernel	Total nitrogen	Alcohol-soluble nitrogen as percent of total nitrogen	Water-soluble nitrogen as percent of total nitrogen	Nitrogen not precipitated by $\text{CCl}_3\text{COOH}$ as percent of total nitrogen
		%	%	%	%
1	Normal	2.56	40.1	12.9	9.1
	Infected	1.28	47.2	47.4	41.0
2	Normal	2.76	39.3	17.2	14.8
	Infected	1.14	44.6	57.3	54.2
3	Normal	2.84	43.4	19.1	15.6
	Infected	1.39	53.5	59.8	54.2

the whole kernel. In order to answer this question infected kernels were dissected into two parts: one entirely normal to all appearance, and the other showing evident signs of infection. Both fractions were then ground separately and the gluten washed out. It was not possible to wash out even traces of gluten from the infected parts of the kernel, whereas, from the undamaged parts, the gluten washed out quite normally, although it was somewhat weak. These experiments show that the secretions injected by the bug do not disperse throughout the kernel but localize in the area of infection. Accordingly, one would suspect that the deep-seated changes in the nitrogenous constituents of the kernel would also be limited to this area.

To verify this suggestion undamaged parts of the kernel as well as those immediately adjacent to the point of attack were excised. These normal and damaged fractions were then ground and subjected to analyses, the results of which are summarized in Table VI.

It is readily apparent that the infected areas of the kernels are lower in total nitrogen than the normal portions and, also, that the nitrogenous constituents are less complex. On the average, approximately one-half of the total nitrogen of the infected portions of the kernels is water-soluble and not precipitable with 2% trichloroacetic acid solution.

Evidence of extensive proteolysis in infected kernels has led to attempts to establish a simple method for diagnosing the amount of damage by the bug; this method is based on color reactions for amino acids, the presence of which might be expected in the infected area.

The experiments of Tokareva (1940) showed that on heating a water extract of infected kernels with Millon's reagent, a red coloration develops in the solution and in the protein coagulum formed during heating; in the instance of noninfected kernels only the coagulum is colored. This difference in behavior may be explained as being due to the enzymatic liberation of tyrosine either in the free form or as a peptide. The ready liberation of tyrosine from protein by proteolytic

TABLE VII  
TYROSINE CONTENT OF NORMAL AND INFECTED KERNELS

Sample No.	Tyrosine <sup>1</sup> after precipitation by 2% CCl <sub>3</sub> COOH		Tyrosine <sup>2</sup> after precipitation by Hg(NO <sub>3</sub> ) <sub>2</sub>		Infected area of kernel
	Normal kernels	Infected kernels	Normal kernels	Infected kernels	
	mg/g	mg/g	mg/g	mg/g	mg/g
1	0.34	0.51	0.00	0.04	—
2	0.50	1.13	0.00	0.04	—
3	0.60	1.80	0.00	0.06	0.09
4	0.58	1.73	0.00	0.05	0.08

<sup>1</sup> Method of Zuverkalow (1927).

<sup>2</sup> Method of Thomas (1921).

enzymes was shown by Northrop and his associates (1939); it is, therefore, quite natural that the extensive proteolysis taking place in the kernel infected by the wheat-bug is accompanied by the accumulation of tyrosine in the free and peptide form. This fact was confirmed by Goldstein, Gorbachevskaya, and Sklovskaya (1940).

More detailed data characterizing the content of tyrosine in the normal and infected wheat kernel were obtained by Kretovich, Bundel, and Pschenova (1943) and are given in Table VII. The amount of tyrosine not precipitated by protein precipitants is much higher in infected than in normal kernels. After precipitating extracts from the kernels by means of a mercury salt which is a very strong precipitant, a much smaller content of tyrosine was found than after precipitation with CCl<sub>3</sub>COOH. This proves that the greater part of the tyrosine

found in the infected kernel belongs to the colloidal peptones and other cleavage products of relatively high molecular weight which are formed during the enzymatic splitting of protein.

What then are the essential reasons for the drastic degradation of gluten in the infected grain? Berliner (1931), having discovered the gluten degradation caused by the wheat-bug, was the first to suggest that this phenomenon is due to the presence of powerful proteolytic enzymes in the infected kernels. However, he was unable to prove this by adding water extracts of infected kernels to normal flour, since the gluten-forming property of the flour was not destroyed. Subsequently, the enzymatic nature of the injuries was suggested by Kosmin and Markin (1934) and by Blagoveshchenskii and Sosiedov (1934).

In checking the activity of water extracts, it was found that they acted weakly on the gluten of normal flour, but more active water extracts were obtained by a prolonged autolysis of ground infected kernels in small quantities of water. It may be presumed, therefore, that there exists a specific relation between the colloids of the infected kernel and its proteolytic enzymes, and that salt solutions [employed by Willstätter and Rohdewald (1932) for the extraction of leucocyte-desmoproteases which are closely associated with cellular structures] must be used to effect more rapid and complete extraction of the proteases.

After numerous experiments it appeared that 0.5% sodium carbonate solution was the most suitable solvent; extremely active preparations were obtained by extracting ground infected kernels for 30 to 60 min. The effect of these extracts on gluten may be illustrated by citing the results of a typical experiment.

Normal and infected kernels, selected from one sample, were ground and then extracted in the ratio of 1 : 5 with 0.5%  $\text{Na}_2\text{CO}_3$  solution for 15 hours at 30°C in the presence of thymol. Doughs were then made with the filtered extracts and a strong flour. After a 30-min rest period in a thermostat, the glutes were washed out and their extensibilities determined by the method of Kosmin (1934) as modified by Kranz (1935). The gluten from the dough made with an extract from the normal kernels had an extensibility of 0.47 cm/min; the gluten from the dough made with an extract from the infected kernels, 31.3 cm/min; and the gluten from the dough made with an extract from infected kernels heated for 15 min at 100°C, 0.10 cm/min. When the doughs were allowed to rest for 3 hours at 30°C, that made with the extract from the infected kernels did not give any gluten. Thus it is apparent that the kernels infected by the wheat-bug contain extremely active proteolytic enzymes which are easily destroyed by heating; in the present experiment, the proteolytic activity of the



extract from infected kernels was 66 times higher than that from normal kernels.

In order to compare the proteolytic activity of water and sodium carbonate extracts of ground wheats containing various types of damage, the extracts were used in making doughs with a flour milled from sound wheat which yielded a strong, elastic gluten. The doughs were allowed to stand and their extensibilities determined by the Kosmin-Kranz method (Kosmin, 1934; Kranz, 1935). The results of these tests, given in Table VIII, show that wheat infected by the wheat-bug

TABLE VIII  
PROTEOLYTIC ACTIVITY OF DIFFERENT TYPES OF WHEAT

Type of wheat	Extensibility of gluten from dough made with the extract	
	Water	Na <sub>2</sub> CO <sub>3</sub>
	<i>cm/min</i>	<i>cm/min</i>
Control <sup>1</sup>	0.01	0.02
Normal	0.04	0.03
	0.03	0.02
	0.03	0.03
	0.02	0.07
100% infected by other insects	0.03	0.01
	0.02	0.06
Germinated	0.05	0.03
	0.01	0.01
	0.04	0.02
	0.07	0.03
	0.09	0.03
Frosted	0.12	0.03
	0.02	0.05
Drouth-damaged	0.05	0.03
	0.04	0.02
	0.04	0.01
	0.03	0.02
	0.02	0.02
100% infected by wheat-bugs	0.76	1.17
	1.25	2.00
	0.50	2.75
	4.20	17.00
	19.20	30.00

<sup>1</sup> Dough made with pure water or 0.5% Na<sub>2</sub>CO<sub>3</sub> solution.

can easily be distinguished from any other type of damage by the high proteolytic activity of both water and sodium carbonate extracts; moreover, in the instance of wheat-bug damaged grain, the water extracts are lower in proteolytic activity than the sodium carbonate extracts. These observations establish beyond doubt that gluten degradation is caused by the high proteolytic activity of wheat in-

fected by the wheat-bug; also, that there are insignificant quantities of soluble proteases in wheat containing other types of damaged kernels.

Having demonstrated the high proteolytic activity of sodium carbonate extracts of infected grain, an attempt was made to accelerate the test. The extract was prepared by adding the sodium carbonate solution, previously heated to 40°C, to the ground wheat and allowing the suspension to stand in a thermostat at 37°C for 30 min. Doughs made with the filtered extracts were left in a thermostat maintained at 30°C for 30 min; the gluten was then washed out with water at 20°C and tested according to the Kosmin-Kranz method. The results of the accelerated technique given in Table IX show a still more

TABLE IX  
PROTEOLYTIC ACTIVITY OF DIFFERENT WHEATS AS MEASURED BY THE  
ACCELERATED TECHNIQUE

Type of grain used for obtaining the extract	Extensibility of the gluten
	<i>cm/min</i>
Control <sup>1</sup>	0.05
Normal	0.03 0.02 0.04 0.03 0.09
Germinated	0.02 0.12
Frosted	0.04
Drouth-damaged	0.05
100% infected by the wheat-bug	195. 116.

<sup>1</sup> Dough made with 0.5% Na<sub>2</sub>CO<sub>3</sub> solution.

striking difference than those recorded in Table VIII. The method may be recommended as a more convenient means for diagnosing infection by the wheat-bug and estimating the proteolytic activity of the grain than procedures based upon the determination of protein cleavage products. At the same time, it reflects the first stages of proteolysis which are of most interest to the baker and which are not measured by many other methods for the quantitative determination of proteolytic activity. The precision of this method could be increased by replacing the Kosmin-Kranz extensibility test with a determination of the physical properties of the dough in an apparatus such as the alveograph or farinograph.

Our experiments with sodium carbonate extracts refute the assumption that the high proteolytic activity of the flour from infected grain

is due to the increased content of sulfhydryl compounds. To secure definite proof that sulfhydryl compounds play no part in the processes of gluten degradation in wheat-bug infected kernels, the quantity of such compounds was determined in the flour from infected and normal kernels by a modification of the method of Woodward and Fry (1932). The results showed that the high activity of proteinases in infected kernels could not be explained by an increased content of sulfhydryl compounds.

Since it has been determined that the degradation of gluten in the infected kernel is due to the presence of extremely active proteolytic enzymes, it is appropriate to study the conditions of their activity, especially in relation to pH and temperature. The methods generally used for determining proteolysis, based on the determination of free amino groups, could not be effective in the present case as they do not

TABLE X  
EFFECT OF pH ON PROTEINASE ACTIVITY OF INFECTED WHEAT

pH	Nitrogen dissolved by enzyme action <sup>1</sup>	
	Test 1	Test 2
	mg	mg
3.2	0.46	1.44
4.2	2.94	—
5.2	3.46	4.33
6.2	4.55	—
7.2	4.89	4.75
8.0	4.01	—

<sup>1</sup> Per 5-ml solution.

record the action of enzymes during the first stages of proteolysis. The following method was therefore employed: 9 ml of glycerol extract (40% glycerol) from ground infected kernels was added to 0.5 g of finely ground vacuum-dried gluten; the pH of the mixture was brought to the desired value with 10 ml of buffer solution prepared according to McIlvaine (1921). Thymol was used as an antiseptic. Simultaneously, a control experiment was carried out under the same conditions, using 9 ml of 40% glycerol. The experimental and control tubes were placed in a thermostat at 36°C for 16 hours. The total nitrogen in 5 ml of the filtered solution was then determined, and from this the total nitrogen of the corresponding control and of the enzyme extract was deducted. The resulting value served as a measure of proteinase activity. Gluten, rather than edestin, gelatin, or other proteins, was used as a protein substrate since the researches of Northrop (1939) suggest that the activity of the enzyme on various proteins would be different. The results of two typical experiments, given in Table X,

show that a neutral or slightly alkaline reaction is most favorable for the action of the gluten-dissolving enzymes of infected wheat. More striking results can be obtained if the measurement of proteolytic activity is based on the effect of the solutions on gluten extensibility; very evident by this technique are the greater activity in an alkaline medium and the considerable decrease in activity upon acidification of the dough.

Experiments to determine the effect of temperature showed, as anticipated, that the proteinase activity decreased with the lowering of the temperature.

To obtain dry preparations of the proteolytic enzymes and to investigate their properties, sodium carbonate extracts of normal and infected kernels, obtained according to the method described above, were dialyzed against distilled water until all dialyzable substances were removed. The liquid in the collodion sack was saturated with a tenfold volume of ethanol and then acidified with several drops of 10% acetic acid solution until an abundant precipitate was obtained. The precipitate was washed several times with strong alcohol, then with anhydrous ether, and dried in a vacuum. The preparations were light-grey powders; the yield from normal kernels was 1.48% and from infected kernels, 1.88%.

When preparations from infected kernels amounting to 0.1% were added to flour having good gluten, the extensibility of gluten washed out of the dough after a 30-min rest period at 30°C increased from 2.25 cm/min to 24.0 cm/min. When 1.0% of this preparation was added to the same flour, it was impossible to wash gluten from the

TABLE XI  
EFFECT OF DRIED ENZYME PREPARATIONS ON GLUTEN OF STRONG AND WEAK FLOURS

	Gluten extensibility	
	Strong flour	Weak flour
	<i>cm/min</i>	<i>cm/min</i>
Control	0.07	0.62
Control + 0.1% of preparation from normal grain	0.12	0.94
Control + 0.1% of preparation from infected grain	4.26	Gluten did not wash out

dough at the end of the rest period; a sticky incoherent mass, characteristic of doughs made from flours prepared from infected wheat, was obtained.

It was of special interest to investigate the effect of these dried enzyme preparations on the physical properties of gluten of strong and

weak wheat flours milled from sound wheat. Doughs containing 0.1% of the dry enzyme preparations from normal and infected wheat, respectively, were prepared with the two flours; after allowing them to stand at 18° to 20°C for 3 hours, the gluten was washed out and tested according to the Kosmin-Kranz method. The results, recorded in Table XI, are very significant and demonstrate the fallacy of estimating the quality of infected grain by recording the percentage of infected kernels in a given blend, as well as the impossibility of establishing any safe maximal infection applicable to every type of wheat.

To study directly the influence of infected grain on the deterioration of glutens of varying quality, 0.5, 1.3, and 6% of infected kernels were added to four wheats, designated as Nos. 1, 2, 3, and 4 in order of decreasing strength. The wheats were then ground and doughs were prepared. After the doughs had rested at 30°C for 30 min, the glutens were washed out and their extensibilities determined by the Kosmin-Kranz method under strictly comparable conditions.

The results, given in Table XII, clearly show that the initial quality of the wheat is the most important factor in relation to the damage

TABLE XII  
INFLUENCE ON GLUTEN QUALITY OF THE ADDITION OF INFECTED KERNELS TO  
WHEATS OF VARYING STRENGTH

Sample No.	Wheat type	Gluten extensibility				
		Percentage of infected kernels				
		0%	0.5%	1%	3%	6%
		cm/min	cm/min	cm/min	cm/min	cm/min
1	Strong	0.01	0.01	0.02	0.30	2.3
2	Intermediate	0.03	0.03	0.25	0.80	2.5
3	Fairly weak	0.06	0.90	0.90	13.40	41.1
4	Weak	0.30	2.10	2.70	20.70	50.0

caused by the wheat-bug; even after the addition of 6% of infected grain, the glutens from the two strongest wheats (Nos. 1 and 2) were normal, whereas those from the weakest wheats (Nos. 3 and 4) were originally poor and could only be washed out with great difficulty. From the standpoint of baking quality, it is obviously illogical to classify wheat solely on the basis of the percentage of infected kernels since one sample containing a low percentage of infected kernels may give poor bread, whereas another sample will produce good bread even though it contains a relatively large percentage of infected kernels. The extent of damage caused by any particular degree of infection depends upon a number of factors, such as the species of wheat-bug,

the stage of maturity at which the grain is attacked, the kind of wheat, and the original quality of the gluten.

It must be remembered that there are other insects which, like the wheat-bug, pierce the maturing kernels and produce pale areas and dark spots but which do not influence the quality of the gluten, dough, and bread. An example is the larvae of *Haplothrips tritici*, as recently reported by Grivanov (1938). We have examined a series of wheat samples which contained from 10 to 28% of infected kernels and yet had good gluten and made excellent bread; it is quite plain that the wheats were not punctured by harmful bugs.

It follows from the facts which have been cited that the only reliable method of estimating the quality of grain suspected of damage by the wheat-bug is to determine gluten or dough quality. At the present time such an estimation can be made most precisely with the help of Brabender's farinograph or Chopin's alveograph. As the disintegration of the dough takes place only after it has rested, it is necessary to record the farinograms and alveograms immediately after the dough is made and again after it has stood in a warm place for 60 to 90 min. A similar method of estimating the degree of grain infection by the wheat-bug, based on the use of Chopin's extensimeter, was adopted in France and legalized by a special regulation ("Office National Interprofessionnel du Blé," 1937). Price discounts for infected grain have been established in conformity with the degree of damage, called "virulence commerciale."

By what means is it possible to improve wheat damaged by the wheat-bug? In the studies of Berliner (1936), Křanz (1935), and others, there are numerous indications of the favorable effect of heat treatment on the quality of damaged wheat. We can fully confirm these indications; the heating of moistened grain with warm air gives good results, improving the quality of the gluten and the dough. Beneficial results are also obtained by adopting the method of Weickmann (1938) who used hot water. However, the most convenient and suitable method is the treatment of grain by steam. Upon treating wheat containing 32% of infected kernels with steam for 1.5-3.0 min, a normal gluten was obtained. It is presumed that the treatment of wheat with steam could be recommended not only for damaged grain but also as a simple method of conditioning normal wheat.

### Summary

Studies of the biochemical properties of wheat which has been punctured by certain species of wheat-bugs show that such infection somewhat lowers the total nitrogen and considerably increases the

proportions of alcohol-soluble and water-soluble nitrogen, especially the latter. In wheat containing 10-15% of kernels punctured by the wheat-bug, the gliadin content of the gluten is substantially increased. Gliadin solutions prepared from badly damaged kernels are characterized by a lower viscosity and optical activity than normal; the gliadin has a higher total sulfur content, but the sulfhydryl compounds of the glutathione type are not increased.

Flour from infected wheat differs from normal in possessing higher titratable acidity, higher amylase activity, and a markedly greater proteolytic activity. Extensive proteolysis, with the accumulation of water-soluble nitrogen and of nitrogen not precipitable by trichloroacetic acid, occurs during the autolysis of flour milled from infected wheat.

Damage caused by the wheat-bug, at least when the wheat is attacked during later stages of maturation, is localized in the region of the puncture. Endosperm dissected from the damaged areas of kernels is considerably lower in nitrogen content than that of the undamaged areas, and approximately one-half of the total nitrogen is water-soluble and not precipitable with trichloroacetic acid solution. Damaged kernels contain an increased quantity of tyrosine, which serves as a rapid and convenient method of detecting the presence of infected kernels.

Proteinases of the infected wheat are most active in neutral or faintly alkaline solution. Their activity is greatly reduced by moderate acidification.

Water extracts of infected kernels influence the gluten of normal flour only slightly, whereas extracts obtained with 0.5% sodium carbonate solution rapidly destroy the gluten but lose their activity on heating. Dry enzyme preparations can be prepared from infected kernels; when 0.1% is added to normal flour, the gluten-forming properties are rapidly destroyed. Aqueous solutions of the dry enzyme preparations are completely inactivated by heating.

These studies prove that the destruction of gluten in infected wheat is caused by active proteolytic enzymes injected into the kernel by the wheat-bug. The harmful effect of an admixture of damaged flour or of enzyme preparations upon the quality of normal flours is much less for strong than for weak flours. It is therefore impossible to set a maximal allowable percentage of infected kernels which would be applicable to every type of wheat.

Normal gluten can be obtained from wheat containing up to 32% of infected kernels after treatment with steam for 1.5 to 3.0 min.

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## pH VARIATIONS IN SODA CRACKERS AND THEIR CONTROL WITH DIAMMONIUM PHOSPHATE

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(Presented at the Annual Meeting, May 1942; received for publication May 18, 1942)

The maintenance of a uniform pH in soda crackers has long been a perplexing problem in the biscuit and cracker industry. Baking soda requirements may vary from day to day, and as yet no reliable method has been devised that will predetermine these variable requirements. Johnson and Bailey (1924) suggested a titration procedure for determining soda requirements, but this method was never widely accepted by the industry because it did not provide a complete solution to the problem. One technical shortcoming of this procedure was that it failed to control the variables occurring after the soda had been added at the dough make-up stage. These authors concluded that although a small excess of soda over that required for neutralization is desirable, crackers of excessive alkalinity often result if this excess is not accurately controlled. This was largely due, they believed, to the conversion of the excess bicarbonate to the more highly alkaline normal carbonate during baking. Micka (1941), in a later

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study of the soda requirements of cracker flours, concluded that the excess or "free" soda, as he called it, remained as bicarbonate during the baking, thus ruling out its conversion to the normal carbonate as a possible cause of increased alkalinity in soda crackers.

The investigation reported here was undertaken to determine the nature of pH variations in soda crackers and the possible control of these variations by means of chemical buffering agents.

### Methods

The following experimental formula and procedure were used except where otherwise noted.

	Sponge	Dough
	g	g
Cracker flour	120	80
Yeast	1	—
Salt	—	2
Shortening	—	22
Baking soda	—	Variable
Water	50	23
Fermentation time	18 hours	5 hours
Fermentation temperature	80°F (26.7°C)	80°F (26.7°C)

To facilitate mixing and promote fermentation the sponges were made up on a 500-g flour basis and then subdivided at the dough make-up stage. Sponges were mixed in a McDuffee bowl only long enough to obtain a coherent dough, whereas the doughs were mixed 6 minutes at low speed, using the 3-quart bowl and the cake paddle of a Hobart mixer. After fermentation, the doughs were run through a sheeter three times, then cut, docked, and baked on a steel plate in a gas-fired oven at 500°F (260°C) for 4½ minutes. All pH determinations were made electrometrically, using approximately 20 g of dough or 15 g of crackers with 100 ml of water. Doughs were ground with water, whereas the crackers were powdered before adding the water.

### Results

The pH data presented in Figure 1 were obtained from a series of soda-cracker baking tests in which the quantity of bicarbonate had been varied from 0 to 0.8%. The shape of the curve shows that in a pH range between 6.75 and 8.75 very large pH increases were obtained with small increments of bicarbonate. This observation is of commercial significance because it is this range with which the manufacturer of soda crackers is concerned.

The magnitude of these pH variations is more strikingly shown in Figure 2 which presents data obtained on sponges and doughs, and on crackers in which the baking periods were varied. As the amount of bicarbonate is increased and the pH approaches the critical range, there

is very little effect on pH during the baking. However, when the bicarbonate is increased sufficiently to bring the dough to pH 7.0 or slightly higher, a proportional increase in the pH of the dough results, but an abrupt shift towards greater alkalinity occurs during baking. This high pH persists with little change during a normal baking period of  $4\frac{1}{2}$  minutes, but tends to drop as the baking is extended. Thus there is a lowered alkalinity in overbaked crackers.

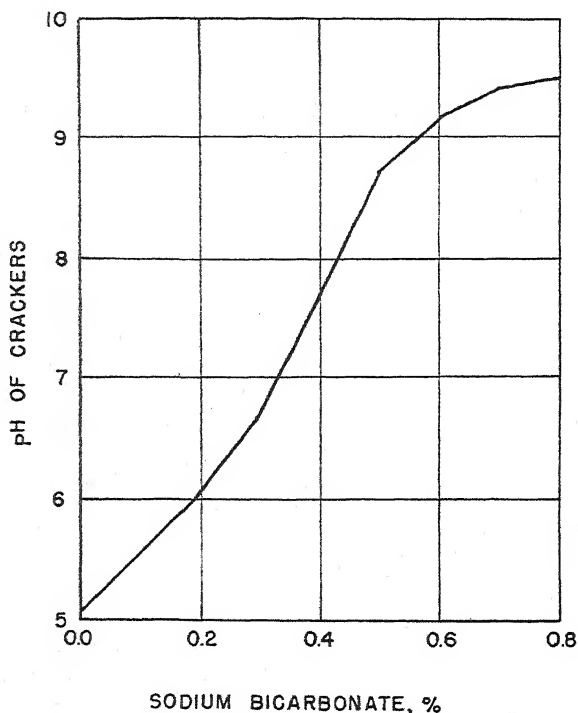


Fig. 1. Effect of increasing amounts of sodium bicarbonate on pH of soda crackers.

In further studies with varying baking periods, normal sodium carbonate and sodium hydroxide were used as neutralizing alkalies in such quantities as to provide the sodium-ion equivalent of the bicarbonate. The normal carbonate values for doughs and crackers, as shown in Figure 3, almost duplicated those of the bicarbonate. The sodium hydroxide curves in Figure 2 were considerably higher in pH immediately after dough make-up, but during dough fermentation they dropped to the approximate level of the bicarbonate doughs and then paralleled the pH of those doughs during the baking.

These studies were then extended to include tests with doughs and crackers containing excess amounts of the three alkalies: sodium bi-

carbonate, normal sodium carbonate, and sodium hydroxide. The same formula was used as before except that the yeast was increased to 1%, and a small amount of sodium sulfite (0.0125%) was added to facilitate handling of the doughs. The three alkalies were varied from none up to a sodium-ion equivalent of 2.5% sodium bicarbonate. A straight-dough procedure with a  $2\frac{1}{2}$  hour fermentation was used.

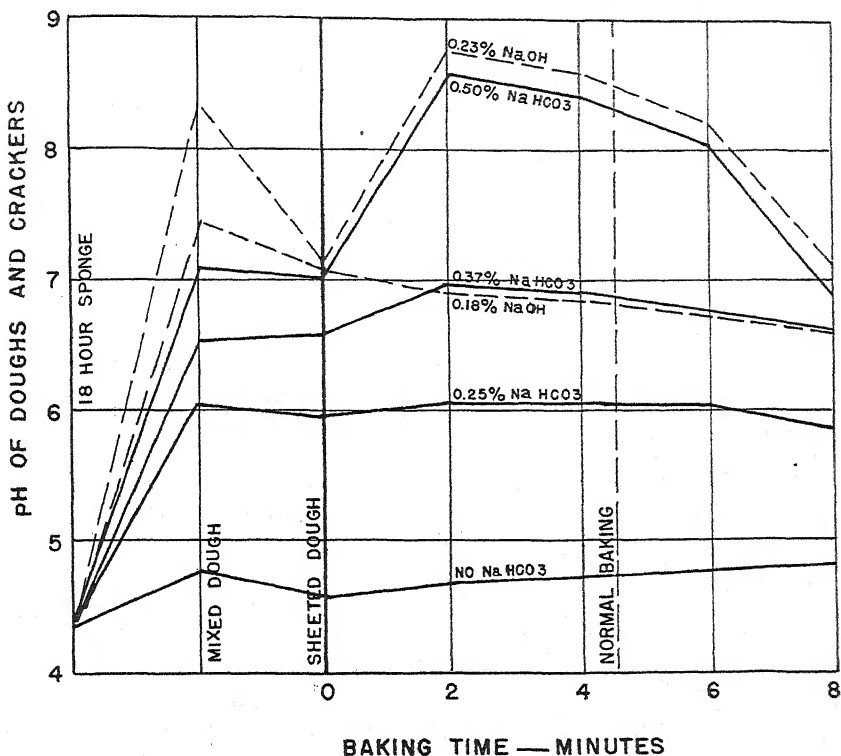


Fig. 2. Effect of increasing amounts of sodium bicarbonate and sodium hydroxide on pH of sponges, doughs, and crackers at variable baking periods.

The pH was followed in both the doughs and the baked crackers. The curves presented in Figure 4 show a wide spread in pH between the dough and cracker made with sodium bicarbonate and much smaller spreads when the normal carbonate and sodium hydroxide were used. Upon baking, the pH of the doughs containing carbonates increased whereas that of the doughs containing sodium hydroxide decreased. Each of the three alkalies tends to affect the pH of the dough in proportion to its specific alkalinity. However, in the baked cracker the pH of both the "bicarbonate" and "hydroxide" crackers tends to approximate that of the crackers made with normal carbonate.

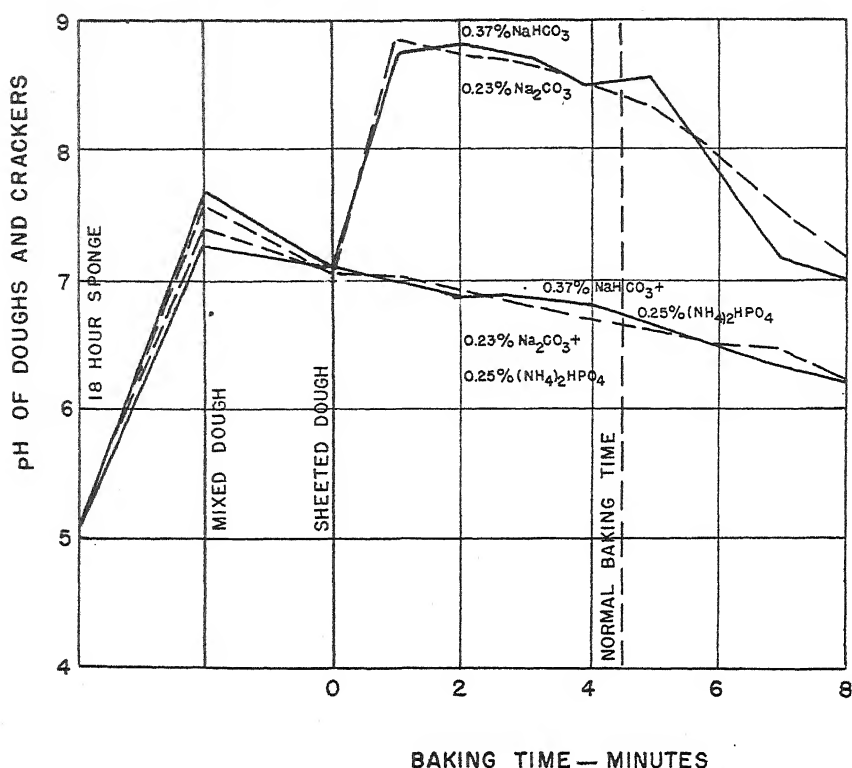


Fig. 3. Effect of diammonium phosphate in controlling pH of soda crackers at variable baking periods when using sodium bicarbonate or the normal carbonate as neutralizing agents.

It was of interest also to ascertain whether carbon dioxide remained in the crackers made with the largest amounts of the three alkalis. The analyses are given in Table I. The recovery of carbon dioxide

TABLE I  
CARBON DIOXIDE IN FINELY PULVERIZED CRACKERS

Alkali used	Alkali added	Carbon dioxide evolved <sup>1</sup>	Approximate theoretical carbon dioxide recovery
	%	ml	%
Control	0.00	5.0	—
Sodium bicarbonate	2.50	21.5	25
Normal sodium carbonate	1.56	22.5	52
Sodium hydroxide	1.18	13.0	—
Control with added sodium bicarbonate <sup>2</sup>	2.50	71.0	100
Control with added normal sodium carbonate <sup>2</sup>	1.56	38.5	100

<sup>1</sup> Determined as outlined in Cereal Laboratory Methods (1941), p. 171.

<sup>2</sup> Powdered carbonates were weighed directly into the pulverized crumb of the control crackers before analysis.

from carbonates added directly to the pulverized cracker-crumb control was the basis for calculating the carbon dioxide which remained in the crackers containing the alkalis. These data suggest that the excess bicarbonate does not remain as such in the baked product.

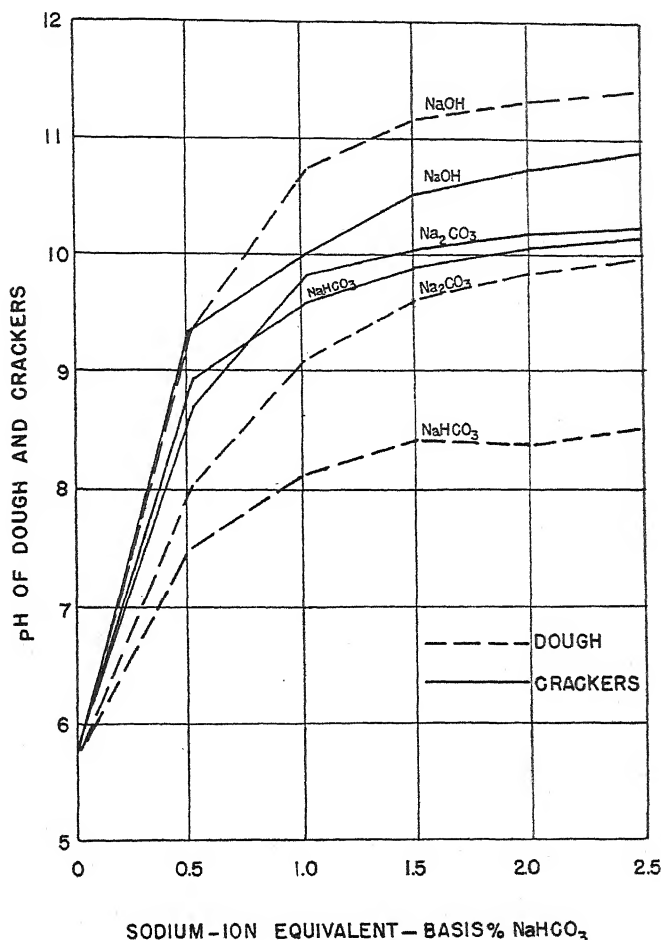


Fig. 4. Effect of excessive amounts of sodium bicarbonate, normal carbonate, and sodium hydroxide on pH of doughs and soda crackers.

However, the partial recovery of 52% of the carbon dioxide which was added to the dough as the highly alkaline normal carbonate, and the gain in the carbon dioxide content of crackers made from doughs which received sodium hydroxide, indicate that mass reactions may be involved in the baking-out of cracker doughs. These reactions probably involve the formation and breakdown of alkali-protein-acid complexes.

The commercial production of soda crackers has advanced to a high degree of efficiency. The various operations, such as mixing, fermentation, machining, are well controlled in most cracker plants, yet pH variations may occur and result in an inferior product. Any buffering agent which could be added to the dough in reasonable amounts and which would maintain the pH of the finished crackers within a desirable range would be of great value to the industry.

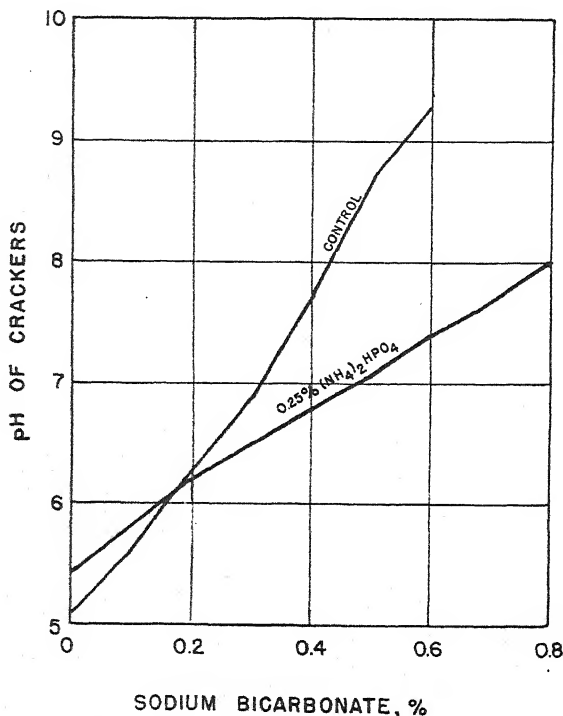


Fig. 5. Effect of diammonium phosphate in controlling pH of soda crackers made with increasing amounts of sodium bicarbonate.

Most of the common buffering salts, such as the acetates and phosphates of sodium and calcium, do not satisfactorily control the pH when used in practicable amounts. However, diammonium phosphate was found to be unusually effective. The addition of 0.25% diammonium phosphate to soda-cracker doughs in which the bicarbonate was used in increasing amounts held the pH of the crackers within relatively narrow limits (Fig. 5). Diammonium phosphate also prevents the alkaline shift which results from an excess of alkali over that required to neutralize cracker dough to pH 7.0 (Fig. 3). Furthermore, as shown in Figure 6, diammonium phosphate buffers on the

acid side. In general, however, acid crackers are not so troublesome in commercial production as are alkaline crackers.

The manner in which this ammonium salt tends to control the pH in soda crackers is unique. It is essentially a neutral salt and when mixed in a neutral dough apparently does not decompose to any appreciable extent. However, if excessive bicarbonate is present, the alkali formed during baking (Fig. 2) immediately tends to react with

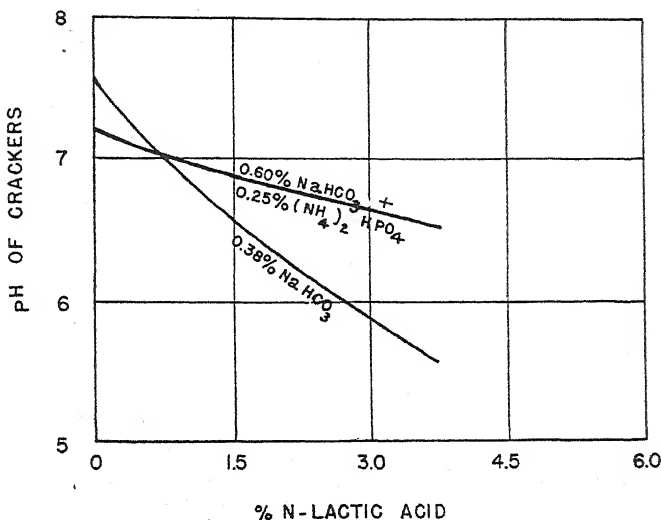


Fig. 6. Effect of diammonium phosphate in controlling pH of soda crackers on the acid side.

the diammonium phosphate, thereby causing ammonia to be driven off and phosphoric acid to be released; this acid, in turn, neutralizes the alkalinity. The sodium phosphates which remain as residual salts are well known for their buffering action. If, on the other hand, excess bicarbonate is not present, a shift to the alkaline side does not occur during baking, phosphoric acid is not released, and the diammonium phosphate apparently acts as a buffering salt. However, more complex reactions probably occur in which proteins and other dough constituents take part.

### Practical Aspects

Considering the number of variables which influence the final pH of crackers, it is surprising that the average bakeshop superintendent controls this factor as effectively as he does.

During the 18-hour sponge fermentation, organic acids are formed which must be neutralized by the addition of sodium bicarbonate or "soda," as it is commonly known to the industry. As the average



plant lacks the facilities to determine the soda required by a procedure such as that proposed by Johnson and Bailey (1924), empirical methods nurtured within the industry have served as guides indicating the correct soda addition following long sponge fermentation. As an example, there is the anonymous advice to add 2 oz of soda per barrel of flour per degree Fahrenheit rise in temperature during this fermentation. Most experienced men can make a shrewd estimate of the amount of soda necessary to produce good crackers from the rise in temperature and "feel" of the sponge after 18 or 19 hours of fermentation. Trouble is sufficiently common, however, to justify the dissemination of any information that might be helpful in maintaining a uniform pH from one batch of crackers to another.

The reasons for maintaining a uniform pH at some point between 7.2 and 8.4 are well set forth by Johnson and Bailey (1924). Proper texture, flavor, and appearance all depend largely upon accurate pH control. Commercially, soda crackers must be held within these pH limits if they are to be of acceptable quality and thus hold a strong place in their competitive field. The difficulties encountered in estimating the optimum addition of soda are indicated in Johnson and Bailey's observations that the soda requirements ranged from 7.75 lb to 9.75 lb of soda per 5 barrels of dough.

Factors involved in pH control from a practical standpoint include: flour characteristics, sponge formula, dough-trough conditions, temperature, humidity, fermentation time, and diammonium phosphate used as a buffer.

During recent years, flour for use in soda crackers has been adapted more closely to its particular function by a better understanding of the requirements and by methods of measuring the qualities desired. Today a survey of sponge and dough flours would probably show a greater difference in analysis between the average sponge flour and the average dough flour than existed 20 years ago. Terms such as "fermentation tolerance," "diastatic activity," and "maltose value" are all comprehended by the cracker baker when he observes that the "sponge rose high enough" and "feels normal." The influence of a flour from a well-blended wheat mix upon pH is usually fairly constant within a given shipment, but the experienced cracker baker notes a variation in pH when shifting from one brand of flour to another or from one shipment to another.

The proportion of flour to water in the sponge affects not only the pH but also the diastatic and proteolytic activity during fermentation. During the first 4 to 6 hours the rate of fermentation is slow; this is generally believed to be a period of hydration of the sponge constituents. The presence of more water in a "slack" sponge naturally

leads to a faster complete "wetting" and usually a more rapid drop in pH during the sponge fermentation. To keep this hydration period as uniform as possible, care should be taken to insure accurate measurement of the water and of mixing time.

One of the most difficult factors to control is the effect of the dough culture which remains on the inner surface of the dough troughs when they are emptied. This adhering dough can stimulate fermentation to such an extent that 10 to 12 oz more soda per 5 barrels of dough are required, even when all other factors are held as uniform as possible. This difference is between the first dough mixed in a trough that has been scraped clean and out of use for two or three days, during which time the culture loses its vitality, and a second mix in the same trough immediately after it has been emptied. In the latter trough, the third mix will require 4 ounces more of soda, and the fourth and fifth mixes will each require additional 2-oz increments. These data are based upon the use of metal troughs. It is the prevailing opinion within the industry that wooden troughs do not show quite such wide variations as metal troughs in the soda requirements for successive batches.

Temperature is the cracker baker's measuring stick. It indicates the point at which fermentation will start and, to a limited extent, when it will be complete. For example, a sponge "set" at 72°F (22.2°C) and fermented in a room held at 81°F (27.2°C) will give a temperature reading of 83°F (28.3°C) after 18 hours. Assuming that 7 lb of soda will give the desired pH of 7.8 in the resulting crackers, how much soda will be required by the next sponge that gives a reading of 82°F (27.8°C) at the end of the fermentation period? Data which the authors have accumulated indicate that, within the range of 8 to 12 degrees rise in temperature during the sponge fermentation, 6 oz of additional soda per 5 barrels of flour are required per degree Fahrenheit rise in temperature; no data are available beyond this temperature range.

In studying the control of the dough-room factors it has been found that 81°F (27.2°C) and 76% relative humidity provide a favorable environment for fermentation. A lower temperature may hinder fermentation and proteolytic activity to such an extent that the crackers will have a pH between 9 and 10 and exhibit a tendency to "buckle" out of shape during the bake. If the relative humidity decreases sufficiently to allow the doughs to crust over during the 3- or 4-hour fermentation after the soda has been added, the consequent physical "holding down" of the dough seems to have much the same effect as a slight drop in temperature.

On the other hand, if a little extra time elapses before baking, the crackers will drop below pH 7.0 with all the attendant ills of "acid" crackers. This undesirable result emphasizes the importance of scheduling plant operations so that the time interval between the setting of the successive sponges equals the baking time of each dough.

To illustrate these factors, assume a dough room at  $81^{\circ}\pm 1^{\circ}\text{F}$  and  $76\%\pm 2\%$  relative humidity. A series of five sponges are set in troughs that were scraped and out of use for three days. The soda requirement under these conditions is 7 lb per 5 barrels for  $11^{\circ}\text{F}$  rise in temperature with a 6-oz differential per degree variation. Doughs 1 and 2 were set at  $72^{\circ}\text{F}$  ( $22.2^{\circ}\text{C}$ ) and rose to  $82^{\circ}\text{F}$  ( $27.8^{\circ}\text{C}$ ) and received 6 lb 10 oz of soda apiece. Doughs 3 and 4 were set at  $73^{\circ}\text{F}$  ( $22.8^{\circ}\text{C}$ ) and rose to  $84^{\circ}\text{F}$  ( $28.9^{\circ}\text{C}$ ) requiring 7 lb of soda apiece. Dough 5 was set at  $71^{\circ}\text{F}$  ( $21.7^{\circ}\text{C}$ ) and rose to  $83^{\circ}\text{F}$  ( $28.3^{\circ}\text{C}$ ) and therefore called for 7 lb 6 oz of soda. The same troughs were used as soon as emptied, and the next day the soda schedule was built around a basis of 7 lb 10 oz of soda per 5 barrels per 11-degree rise with a 6-oz differential per degree variation. To follow through, the third mix would be based upon 7 lb 14 oz of soda, the fourth mix upon 8 lb, and the fifth mix upon 8 lb 2 oz.

Despite precautions and control measures, the vagaries of fermentation are likely to cause trouble. However, these potential snags can be forestalled in large measure by the use of diammonium phosphate as a buffer during the baking process. This versatile chemical tends to bring both acid and too-alkaline crackers back into the acceptable range. The addition of 2 lb of diammonium phosphate and 2 lb of soda above the schedule requirements makes it possible to produce crackers day after day in which the pH will vary only 0.2 or 0.3.

Observations in cases where the dough-room conditions varied from the standards set up indicate that the buffer action is valuable in combating the visible effects of an excess of residual soda. Doughs in which the fermentation is retarded by a low temperature usually have enough residual soda to give the baked crackers a dark brown color or "bloom" associated with a pH at some point above 8.4. This color reaction seems to be much less marked when diammonium phosphate has been added to the dough, even in cases where the excess of soda is sufficient to overcome the buffer action and result in a pH above 8.4.

### Summary

The pH variations encountered in the fermentation and baking of soda crackers have been investigated. When sodium bicarbonate is added in amounts just sufficient to neutralize dough ingredients and acids of fermentation, little or no change in pH occurs during baking.

However, when an excess of bicarbonate above that required for neutralization is added to the dough, a large shift in pH toward the alkaline side occurs upon baking. Diammonium phosphate was found to be unusually effective in preventing these pH shifts. It also buffers crackers on the acid side of neutrality.

Evidence is presented that excessive bicarbonate in a soda cracker dough does not remain in that form during the baking of the cracker. Conversion to the normal carbonate, along with more complex reactions involving proteins and other dough constituents, undoubtedly takes place.

Problems involved in controlling pH in the commercial production of soda crackers are discussed.

#### Acknowledgments

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## THE SHORTENER TOLERANCE OF BISCUIT AND SELF-RISING FLOURS

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(Presented at the Annual Meeting, May 1942; received for publication May 18, 1942)

Several years ago, the Cincinnati section, American Association of Cereal Chemists, appointed a Research Committee to further activity in soft wheat problems. The project which was selected comprised a study of the optimum shortener level in biscuit flours of varying viscosity. This paper presents the studies which were undertaken.

Whiting (1932) used 16% shortening in the work of the A.A.C.C. Subcommittee on Tests for Biscuits and Self-Rising Flours. He suggested that the shortening levels in many biscuit formulas may be too low for optimum results. Walter (1933) employed 13% shortening for soft flours and 15% for hard flours. In more recent studies (Whiting, 1936; Gookins, 1941), shortener levels of the order of 12.5% have been advocated for all biscuit-type flours. In Cereal Laboratory Methods

(4th ed., 1941), 12.5% shortening is specified in the biscuit test for self-rising flours.

The objects of the present study were to determine whether the 12.5% shortening level recommended in test-biscuit formulas is too low and whether the shortener requirements vary with the protein content and viscosity of the flour. Three series of collaborative studies were undertaken, the first in 1936-37, the second in 1939-40, and the third in 1940-41. In the first series, biscuits were baked from three bleached, soft wheat flours selected to cover the viscosity range encountered in commercial self-rising flours, employing corn oil as the shortening agent at levels of  $4\frac{1}{2}$  to  $17\frac{1}{2}$ % in 2% increments. The results of these tests were not sufficiently conclusive to show definitely whether shortener requirements vary with the viscosity of the flour. The strongest flour, however, did not exhibit as much tolerance to variations in shortening level as the two weaker flours. It was the consensus of opinion that the study should be repeated, employing still higher percentages of shortening and adjusting the absorption to compensate for the effects of the varying fat levels on the consistency of the dough.

These suggestions were followed by the 1939-40 Research Committee. Only the second and third series of studies will be detailed here.

### Materials and Methods

*Second Series (1939-40 Research Committee).* Two flours, D and E, which differed widely in protein content and viscosity (Table I) were converted to self-rising flours by the addition of 1.86% monocalcium phosphate, 1.5% powdered sodium bicarbonate, and 1.75% salt. Summer-grade cottonseed oil which had been alkali-refined, deodorized, and lightly bleached was employed as the shortening agent at levels of 10, 15, 20, and 25% (flour basis). Preliminary tests were made to determine the optimum absorptions for each fat level for both flours. Detailed instructions for carrying out the biscuit test were supplied each collaborator. The required amount of water was placed in the mixing bowl, the flour added, and the desired amount of oil distributed over the surface of the flour. Mixing was conducted with a Kitchen Aid mixer for 10 seconds at low speed, the mass cut down, and the mixing continued for an additional 10 seconds at low speed. In other details the instructions essentially corresponded to those now given for the biscuit test in *Cereal Laboratory Methods* (4th ed., 1941). Biscuit volume was determined by seed displacement. The biscuits were judged for grain, tenderness, flavor, and crumb color, according to the scoring system proposed by Walter (1935) and subsequently

included in the latest revision of Cereal Laboratory Methods (4th ed., 1941). Total scores, which include biscuit volume in addition to the quality factors just enumerated, were computed by each laboratory. Since the adverse effect of increased shortening on crumb color would influence the total scores, the total scores omitting crumb color were also computed to obtain a more reliable measure of the effect of shortening level on the other quality factors.

*Third Series (1940-41 Research Committee).* Two shortenings, a lightly bleached cottonseed oil and hydrogenated shortener, each at levels of 13, 15, 17, and 19%, were investigated employing one flour (F,

TABLE I  
CHARACTERISTICS OF FLOURS EMPLOYED IN BISCUIT TESTS

Flour <sup>1</sup>	Protein <sup>2</sup>	Ash <sup>2</sup>	pH	Viscosity	
				No time	1-hr. digestion
D	%	%		° Macm	° Macm
E	10.8	0.36	5.7	148	164
F	8.0	0.40	5.9	57	72
	8.5	0.37	5.2	61	84

<sup>1</sup> Flour D: 92% patent, bleached with Agene and Novadel, milled from a mixture of 30% Illinois hard, 20% low protein Kansas, and 50% Indiana red wheats.

Flour E: 92% patent, bleached with Agene and Novadel, milled from Indiana red wheat.

Flour F: 85% patent, bleached with Beta Chlora and Novadel, milled from Indiana red wheat.

<sup>2</sup> 15.0% moisture basis.

Table I). As in the previous year, the optimum absorption for each fat level was determined by preliminary experiments. For each 1% increase in shortener, a decrease of about 0.4% water was necessary. The method of incorporating the hydrogenated shortener was the same as that given in Cereal Laboratory Methods (4th ed., 1941). In all other respects the experiments were conducted by the methods outlined for the second series.

### Results and Discussion

The 1939-40 studies are summarized in Figure 1, which shows the average biscuit scores for all collaborators for the various shortening levels. At any given shortener level, the softer flour (E) gave the better biscuits. For this flour the optimum shortening level was 15% as compared with 20% for the stronger flour (D). For all practical purposes, however, there is little difference between the optimum shortening level of the two flours since the quality of the biscuits made from the weak flour with 15 and 20% shortening, respectively, did not differ materially. Above 20% shortening, a marked decrease in biscuit

quality occurred with both flours. In Figure 1 the total scores, less crumb color, are also shown. It is noteworthy that increasing additions of the cottonseed oil, which was appreciably pigmented, had a more pronounced effect on crumb color than on the other quality factors.

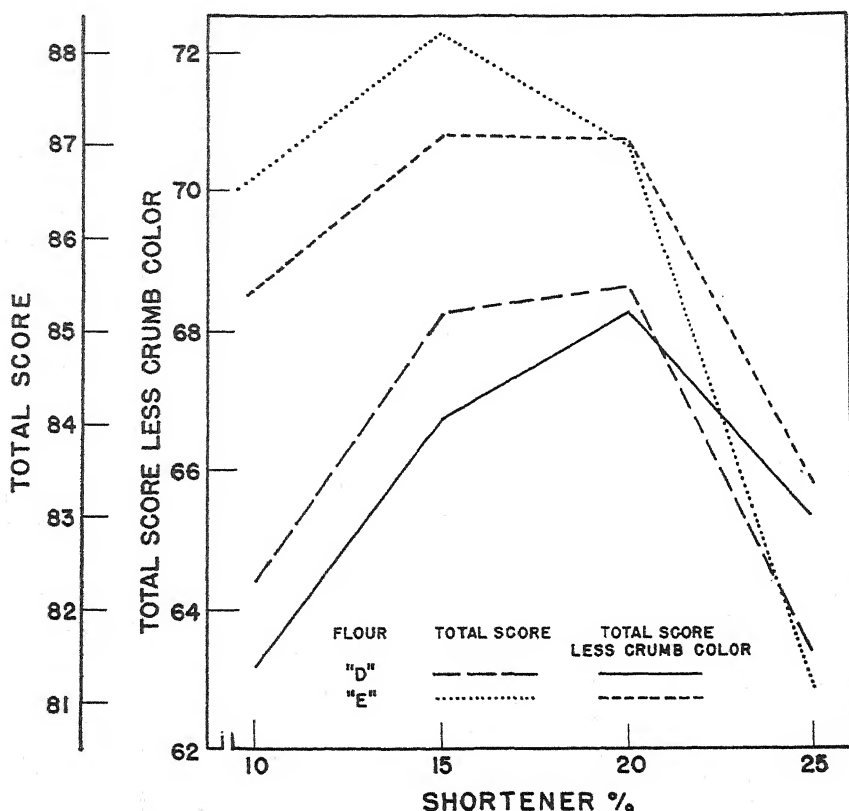


Fig. 1. Effect of varying percentages of cottonseed oil shortener on total score of biscuits from Flours D and E (outer ordinate). The effect of crumb color on total score is also removed for comparative purposes (inner ordinate). (1939-40 Research Committee, Cincinnati Section, A.A.C.C.)

The results of the 1940-41 studies are summarized in Figure 2. The liquid and solid shorteners both gave biscuits of optimum quality when used at the 19% level. This level is in close agreement with that found in the previous year's work.

The optimum percentage of shortening found in these studies is considerably higher than the 12.5% level currently employed in biscuit tests. As the fat content was increased, the specific volume of the biscuits showed little change, but the tenderness of the biscuits was markedly improved; also, crumb grain was slightly better. Although

cottonseed oil had a significant effect upon the crumb color of the biscuits, the decrease in crumb color scores with increasing shortening content of the biscuits was not sufficient to offset the over-all improvement in the other quality factors. This is shown by Figure 2, as the

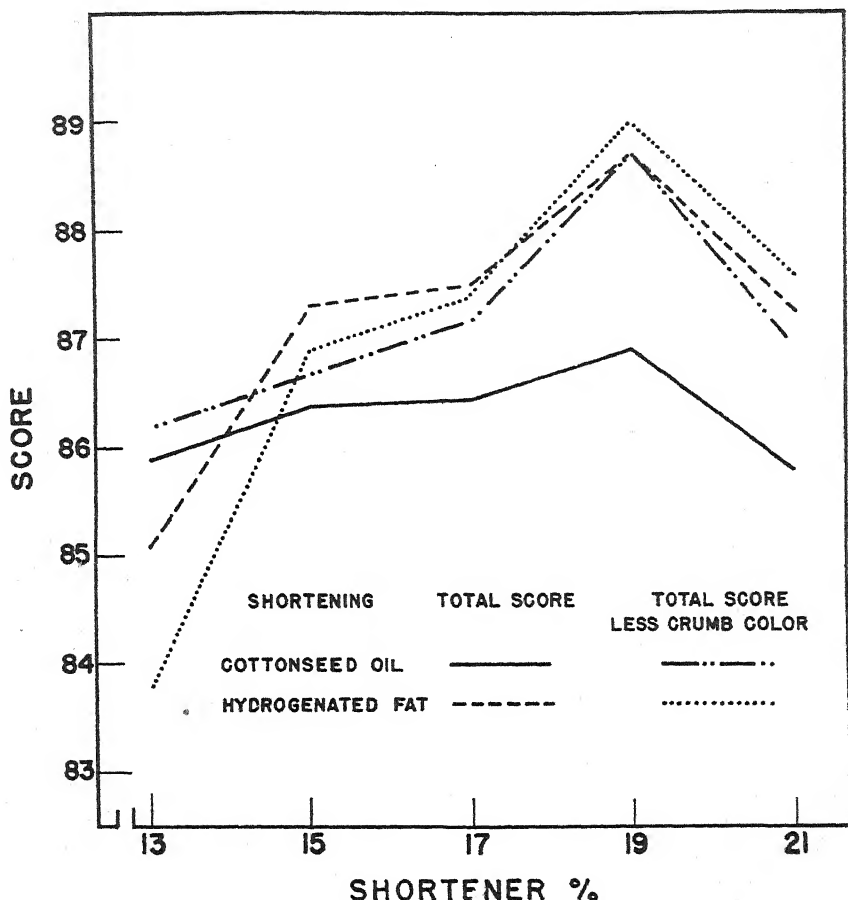


Fig. 2. Effect of varying percentages of cottonseed oil and hydrogenated fat on total score of biscuits. Scores are also shown exclusive of crumb color; these are adjusted for the effect of crumb color on the total score. (1940-41 Research Committee, Cincinnati Section, A.A.C.C.)

cottonseed oil curve (adjusted for the effect of crumb color on the total score) practically coincides with both the adjusted and unadjusted curves for the hydrogenated fat.

### Summary

The optimum shortener level for flours of the type generally supplied for biscuit-making purposes is approximately 19%. Commonly used



test-biscuit formulas specify 12.5% shortening. In employing this high shortening level, the absorption must be decreased to compensate for the effect of the extra fat on dough mobility; a decrease in water absorption of about 0.4% is required for every 1% increase in shortener.

Comparative biscuit tests with a liquid (cottonseed oil), and a solid shortener (hydrogenated shortening) indicated that the optimum shortening level is independent of the type of shortener.

Biscuit flours of varying strength, as indicated by protein content and viscosity, did not differ materially either in their tolerance to varying percentages of shortening or in the quantity required to yield optimum biscuits. However, the softer flours gave the superior biscuits and maximum biscuit quality was obtained with slightly lower percentages of shortening than are needed with the stronger flours.

#### Acknowledgments

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The shorteners were kindly supplied by Procter and Gamble Co. and the Humko Shortener Co. The flours were donated by the Acme-Evans Milling Co. and the Lawrenceburg Roller Mills.

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## THE SULFHYDRYL GROUPS OF WHEAT FLOUR

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In previous studies of the effects of oxidizing agents on fermenting doughs (Sullivan, Howe, Schmalz, and Astleford, 1940), the need for a study of the sulfhydryl groups of the protein and other fractions of wheat flour has been expressed. The following work was done to help fill that need.

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In a study of the sulfhydryl groups of proteins it should be kept in mind that these may exist in at least two well-recognized forms. Some proteins, such as urease, contain free or reactive sulfhydryl or —SH groups. These groups cause the protein to give a positive nitroprusside test when it is in the native form. In some proteins, such as egg albumin, however, there are no free sulfhydryl groups, but after denaturation these groups seem to be liberated so that they give a positive nitroprusside test. These groups are designated as bound or unreactive sulfhydryl groups.

It has been postulated that the improving effects of oxidizing on a dough are due to the oxidation of the sulfhydryl groups of the flour proteins. This oxidation would result in the formation of disulfide cross-linkages between protein molecules. This increased degree of polymerization would therefore seem to produce a dough of greater coherence and gas-retaining properties.

Balls and Hale (1940) have shown the presence of reactive sulfhydryl groups in the petroleum ether extract of flour. Sullivan, Howe, and Schmalz (1936) have demonstrated the presence of glutathione, a tripeptide containing cysteine, in wheat germ. Small amounts of this sulfhydryl-bearing material are undoubtedly to be found in flour as a result of incomplete germ separation during milling.

To our knowledge, no conclusive evidence of the presence of either reactive or unreactive sulfhydryl groups in the proteins of wheat flour has been presented.

### Methods

The presence of reactive sulfhydryl groups in wheat flour proteins was indicated by two tests. The nitroprusside test was made by placing a small amount (approximately 10 mg) of the material on a porcelain spot plate and adding one drop of 1 : 1 ammonium hydroxide and one drop of freshly prepared 5% sodium nitroprusside solution. The development of a pink color on the surface of the material within 30 seconds was taken as an indication of the presence of reactive sulfhydryl groups in the material being tested.

Another more sensitive but less specific test was developed from the work of Anson (1941). He showed that cysteine was the only amino acid which reduced potassium ferricyanide immediately and stoichiometrically in dilute solution at neutral pH. The ferrocyanide formed by this reduction was then converted to Prussian blue which was readily discernible and could be, if desirable, measured quantitatively.

In making this test, 2 ml of the protein dispersion (approximately 2%) was treated with 0.2 ml of 1*M* phosphate buffer (pH 6.8) and

1.0 ml of 0.01*M* potassium ferricyanide. After proceeding for 5 minutes, the reaction was halted by the addition of 0.5 ml of 2*N* sulfuric acid. Then 0.1 ml of the ferric sulfate reagent described by Folin and Malmros (1929) was added. The development of a blue color in the solution was taken as an indication of a reduction of part of the ferricyanide by the protein dispersion, and, in the testing of purified proteins, indicated the presence of reactive sulfhydryl groups. This color could also be measured colorimetrically and converted to its cysteine equivalent from a calibration curve.

To test for the presence of unreactive sulfhydryl groups, the above two tests were applied to the protein material after it had been subjected to treatment intended to denature it.

The nitroprusside test was applied as above after treatment of the proteins with heat, guanidine hydrochloride, or urea. Approximately 10 mg of crystalline guanidine hydrochloride or 15 mg of urea were used in each test.

The ferricyanide test was applied after the addition of 0.5 ml of 10% Duponol PC solution. This is a monosodium sulfate of mixed fatty acids, chiefly  $C_{10}$  to  $C_{18}$ , a synthetic detergent capable of denaturing egg albumin. This test was also applied after heating the protein dispersions both in the presence and absence of this amount of the detergent. Uniformly positive tests in all of these cases were taken as indications of the presence of unreactive sulfhydryl groups in that particular protein.

### The Gluten Proteins

Gluten was prepared from freshly milled unbleached patent flour. The gluten was washed from a flour-water dough, dispersed in 0.005*N* acetic acid, precipitated by the addition of phosphate buffer (pH 6.8) to 0.05*M*. This purified gluten was again dispersed in 0.005*N* acetic acid. Both the dispersion and a sample of the precipitated gluten gave a negative nitroprusside test. The dispersion gave a negative test for the presence of free reducing groups by the ferricyanide technique. From this it was indicated that the gluten proteins did not contain any free sulfhydryl groups.

To test for the presence of any unreactive groups which might be liberated and become reactive during denaturation, the following procedures were employed. Samples of precipitated gluten were heated, treated with guanidine hydrochloride and with urea, and tested with nitroprusside. These tests were uniformly negative. Portions of the gluten dispersion were boiled in the presence and absence of Duponol PC and also treated with Duponol PC at room temperatures. When these dispersions were tested by the Anson ferricyanide technique de-

scribed above, the results were also negative. From this it was concluded that the gluten proteins did not contain unreactive sulfhydryl groups.

Sullivan, Howe, Schmalz, and Astleford (1940) showed that a solution of 2,6 dichlorophenol-indophenol is eventually decolorized when boiled in the presence of gluten. They interpreted this as indicating a reduction of the 2,6 dichlorophenol-indophenol by sulfhydryl groups. Todrick and Walker (1937) employed a similar technique for the estimation of the sulfhydryl groups of egg albumin and claimed it to be specific for those groups.

The results of Sullivan, Howe, Schmalz, and Astleford (1940) were confirmed. It was also found that at boiling temperatures gluten

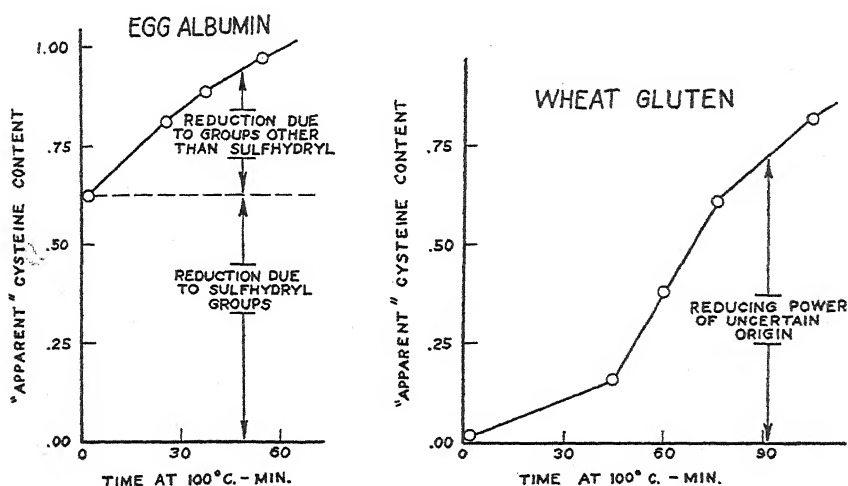


Fig. 1. The reducing power of egg albumin and wheat gluten as a function of time at 100°C.

reduced ferricyanide under the conditions described above. Anson (1939) showed that groups other than the sulfhydryl of egg albumin were able to reduce ferricyanide at boiling temperatures. A sample of egg albumin was prepared after the method of Sorenson with the exception that no attempt was made to obtain a crystalline product. The final solution was dialyzed against distilled water until free of ammonium sulfate. The sulfhydryl content of this protein was determined and found to be equivalent to 0.62% cysteine. Aliquots of this solution and of the gluten dispersion were subjected to the action of ferricyanide in the presence of Duponol PC for various periods at boiling temperatures. The amount of ferricyanide reduced was then estimated colorimetrically and expressed as "apparent cysteine content" of the respective proteins. These results are presented graphically in Figure 1.

While these results alone do not necessarily disprove that the reducing ability of gluten at boiling temperatures is due to sulfhydryl groups, they do support the belief that the action is due to other groups.

### The Water-soluble Proteins

The soluble proteins were isolated from freshly milled unbleached patent flour. One hundred g of flour were digested with 200 ml of distilled water. The dispersion was centrifuged with a Sharples Super Centrifuge. The supernatant liquid was saturated with solid ammonium sulfate and the precipitated proteins separated by filtration. These proteins were redispersed and reprecipitated twice more. The precipitated proteins gave a faintly positive nitroprusside test while in the native state. When dispersed they were able to reduce ferricyanide even without the addition of Duponol PC. These results seemed to indicate the presence of reactive sulfhydryl groups in the soluble proteins. When these proteins were precipitated by the addition of four volumes of alcohol and then redispersed, however, they gave negative responses to both tests. When the filtrate from which these proteins had been precipitated was evaporated to dryness there remained a slight residue of oily material which gave a positive nitroprusside test.

The following experiment was performed to investigate this phenomenon more fully. The soluble proteins were isolated from 1200 g of flour as described above by precipitation from saturated ammonium sulfate. The precipitate was washed with ethyl alcohol and petroleum ether and the washings evaporated under vacuum. The residue consisted of 2 g of fatty material. The nitrogen content of this material as well as the fraction insoluble in acetone is shown in Table I along

TABLE I  
ANALYSIS OF FATTY MATERIAL ISOLATED FROM THE SOLUBLE PROTEINS

Determination	Isolated fraction	Flour fat <sup>1</sup>	Phosphatide <sup>1</sup>
Kjeldahl, % N	1.65	1.65	4.28
Precipitated from acetone, %	20.4	25.0	100.0

<sup>1</sup> Values reported by Sullivan, *et al* (1940).

with similar values reported by Sullivan, *et al* (1940) on representative flour fat and on the phosphatide fraction of flour fat. These analyses indicated that this fraction was similar to whole flour fat and was not especially rich in phosphatides.

That the sulfhydryl reactions of these soluble proteins were due to the presence of contaminating lipids was further confirmed when the

proteins prepared from ether extracted flours failed to give positive nitroprusside or ferricyanide reactions without preliminary denaturation.

After the extraction of lipid material the soluble proteins were dispersed in a small amount of water to give a thick viscous paste. After treatment with guanidine hydrochloride or urea they gave a positive nitroprusside reaction. After treatment with Duponol PC these proteins reduced ferricyanide under the conditions specified above. From these reactions it was concluded that the soluble proteins of wheat flour contained unreactive sulfhydryl groups which were made reactive by denaturation.

### Summary

A preliminary study of the sulfhydryl groups of the various fractions of wheat flour revealed no indications of the presence of either reactive or unreactive sulfhydryl groups in the gluten proteins.

There appeared to be no free sulfhydryl groups in the soluble protein fraction of flour. However, there were definite indications of the presence of unreactive sulfhydryl groups which were liberated on denaturation of these soluble proteins.

The presence of free sulfhydryl groups in the lipid fraction, first demonstrated by Balls and Hale, was confirmed.

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# VARIETAL, STATION, AND SEASONAL EFFECTS UPON SOME PROPERTIES OF MIXOGRAMS MADE FROM HARD RED SPRING WHEAT FLOURS BY VARIOUS MIXING METHODS<sup>1</sup>

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(Received for publication August 13, 1943)

In evaluating the quality of wheat varieties it is exceedingly important that new varieties be compared with well-known wheats acceptable to the milling industry. It is evident that the greater the number of different quality comparisons made, within reasonable limits, the better will be the chances of finding an improved variety. Mixograms furnish an entirely different basis for judging wheat quality from that of protein content, loaf volume, ash, etc., and appear to supply valuable supplemental information regarding that elusive property of wheat known as "quality."

As no recent review is available, it is advisable to summarize those mixograph investigations that deal directly with wheat variety evaluation. Swanson (1939) postulated that the mixogram properties most closely related to baking results were: steepness or ascending slope of the curve; dough development time or time to reach the peak; the character of the peak, whether sharp or rounded; and the general width and height of the curve. It was pointed out that the mixogram characteristics of different wheat varieties should not be compared unless the wheats are grown under comparable environmental conditions and have approximately the same protein content. Varietal patterns were clearly visible among the curves obtained. Varietal differences have likewise been noted by Larmour, Working, and Ofelt (1939) and Ofelt and Sandstedt (1941).

Sandstedt and Ofelt (1940) thought that both baking and mixograph studies made at a uniform protein level were more informative than if made at the original flour protein content. The effect of protein content on curve height was pointed out. Swanson (1940) also commented on the effect of protein content on curve height and on the ascending and descending slopes of the curve. He later (1941) showed that dilution of flour with starch to a common protein level reduced the curves within a variety to the same pattern, but did not obscure the characteristic differences between varieties. The effect of water absorption upon height was found to be less than that of protein content.

<sup>1</sup> Published with the approval of the Director of the Experiment Station.

Johnson, Swanson, and Bayfield (1943) thought the greatest value of mixogram data lay in supplementing baking results. Relative mixing requirements, mixing tolerance, and varietal curve properties, could be derived from the mixograms. Significant relations between protein content and various mixogram characteristics were found. These researchers concluded that protein content was a more likely index of loaf volume than any curve property considered in the study. Swanson and Andrews (1943) stated that the varietal pattern of mixograms was, for all practical purposes, determined by the gluten protein and not by the starch.

Descriptions of mixogram diagrams, with indicated methods of measurement of the more important characteristics, were published by Swanson and Johnson (1943) and by Harris, Sibbitt, and Banasik (1943).

The investigations to be described were undertaken to determine the influence of certain factors on stage of dough development, range of stability, and curve height; they are, in general, a continuation of the work reported by Harris, Sibbitt, and Banasik (1943). The specific objectives were (1) to ascertain if significant differences in mixogram properties exist between varieties and between environments, and (2) to ascertain whether varietal and station differences are inherent in the gluten or starch component of the wheat. The effect of mixing method was also studied.

### Materials and Methods

In the first series, 32 straight grade experimental flours milled from eight varieties (see Table II) of hard red spring wheat, grown under comparable conditions at four stations in North Dakota in 1942, were investigated. A second series represented six of the varieties of series one from the same four stations, but grown in 1941 and 1942.

The milling was done on an Allis-Chalmers mill in a room held at approximately 70°F and 65% relative humidity. A National micro-recording mixer (mixograph) was used; its head had a speed of 89 rpm, and the spring setting was 9. The apparatus was enclosed in an air-conditioned case held at 30°C  $\pm$  3° and 80% relative humidity.

Flours were mixed with distilled water or with the ingredients of the malt-phosphate-bromate formula. The required quantity of liquid, as determined by an experienced operator when baking the flour, was added to 25 g of flour (13.5% moisture basis), and the mixer was run from 8 to 10 minutes, depending upon the variety under test. In using fermented doughs, 50 g of flour was mixed with the malt-phosphate-bromate ingredients in the Hobart equipped with special dough hooks. The doughs were then divided into equal portions by



weight and fermented for 3 hours at 30°C and 75% relative humidity. Punches were made after 105 and 155 minutes of fermentation, respectively. After 3 hours one dough was taken from the fermentation vessel, placed in the mixograph bowl, and a 5-minute curve made, while the other was baked.

The starch used for diluting the flours to a constant protein level, and the dried gluten used in the gluten and starch blends, were prepared by the procedure described by Harris and Sibbitt (1941). The flours were diluted with starch to a protein level of 12%, while the gluten

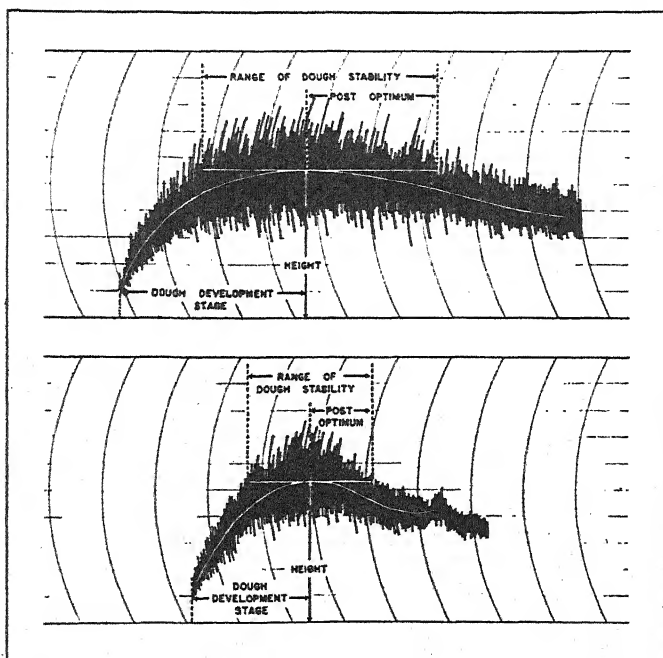


Fig. 1. Representation of the method used in securing the mixogram property measurements. Upper curve was made from a flour with long mixing requirements and range of dough stability. Lower curve was from a flour having an exceptionally short dough development stage and poor dough stability.

and starch blends were at a level of 13.5% (13.5% moisture basis). In the present work gluten-starch blends signify different glutes blended with a constant starch substrate, while conversely starch-gluten blends were made with variable starches blended with a constant gluten base. Moisture content was determined by drying in an electric oven at  $130^{\circ}\text{C} \pm 2^{\circ}$  for 1 hour. The protein was found by the Kjeldahl-Gunning method using 1 g of flour.

Figure 1 shows the method followed in securing the measurements which are similar to those described by Harris (1942). "Dough de-

velopment stage" may be somewhat of a misnomer for remixed doughs following fermentation, but it has been used to avoid confusion.

### Results and Discussion

The influence of several factors on mixogram properties is shown in Table I. The malt-phosphate-bromate formula lengthened the dough development stage and possibly increased the curve height, while fermentation markedly reduced the length of the dough de-

TABLE I  
MEAN VALUES OF MIXOGRAM PROPERTIES FROM THE VARIOUS SERIES  
OF DETERMINATIONS MADE ON THE DIFFERENT SAMPLES

Description of series	Dough development stage	Range of stability	Post-optimum stability	Curve height
	<i>cm</i>	<i>cm</i>	<i>cm</i>	<i>cm</i>
Flours, original protein content, flour-water formula	4.59	4.97		7.32
Flours, 12% protein level, flour-water formula, no fermentation	4.53	5.07	2.56	7.01
Flours, 12% protein level, malt-phosphate-bromate formula, no fermentation	6.39	5.04	2.76	7.86
Flours, 12% protein level, malt-phosphate-bromate formula, fermented and remixed	2.89	3.89	1.82	8.15
Flours, 12% protein level, flour-water formula, no fermentation				
1941 crop	5.17	3.86		5.46
1942 crop	5.11	4.07		5.93
Gluten-starch blends, malt-phosphate-bromate formula, no fermentation	4.13	4.54	2.69	8.47
Gluten-starch blends, malt-phosphate-bromate formula, fermented and remixed	2.65	3.46	1.87	8.71
Starch-gluten blends, malt-phosphate-bromate formula, no fermentation	5.06	4.38		8.11
Starch-gluten blends, malt-phosphate-bromate formula, fermented and remixed	3.35	3.49		8.60

Note: Thirty-two samples were used in all comparisons except for between years, where 24 were employed.

velopment stage and range of stability, and apparently tended to increase curve height. Little difference is apparent in the results between the flours of the 1941 and 1942 crops. In both of the gluten-starch blends, fermentation reduced dough development stage and range of stability in the same manner as for the original flours.

Table II presents the varietal averages of the four mixogram properties from the diluted flour and gluten-starch blend doughs of the 1942 crop. Differences are apparent among varieties in dough

TABLE II

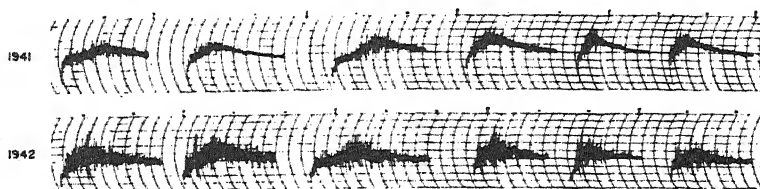
COMPARATIVE VARIETAL AVERAGES OF MIXOGRAM PROPERTIES FROM FLOUR AND GLUTEN-STARCH BLEND UNFERMENTED DOUGHS MADE WITH MALT-PHOSPHATE-BROMATE FORMULA  
(Arranged in increasing order of flour dough development stage)

Variety	Dough development stage		Range of stability				Curve height	
			Total		Post-optimum		Flour <sup>1</sup>	Blend <sup>2</sup>
	Flour <sup>1</sup>	Blend <sup>2</sup>	Flour <sup>1</sup>	Blend <sup>2</sup>	Flour <sup>1</sup>	Blend <sup>2</sup>		
	cm	cm	cm	cm	cm	cm	cm	cm
2822	2.90	2.23	3.31	2.60	1.65	1.32	8.05	8.59
Pilot B	3.56	2.65	3.64	3.33	1.71	1.76	8.08	8.73
Renown	3.99	2.69	3.55	3.41	1.69	1.86	8.04	8.59
Regent	4.33	3.03	4.45	3.90	2.28	2.18	7.89	8.54
2829	4.64	3.33	4.39	3.83	2.01	2.30	7.73	8.25
Thatcher	4.90	4.29	4.44	4.45	2.35	2.66	8.10	8.55
Rival	5.55	4.26	5.44	4.85	3.01	2.82	8.18	8.68
Vesta	7.23	4.64	6.56	5.66	3.63	3.31	8.03	8.81

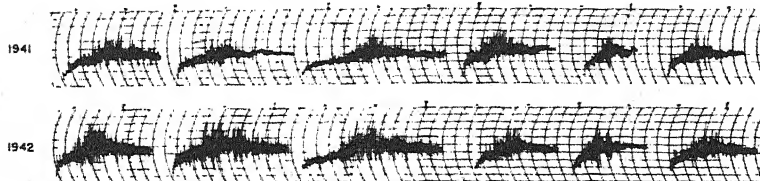
<sup>1</sup> Diluted flour at 12.0% protein level (13.5% moisture basis).

<sup>2</sup> Glutens prepared from the various wheat varieties and blended with a constant starch substrate at a uniform protein level of 13.5% (13.5% moisture basis).

FLOUR AT ORIGINAL PROTEIN CONTENT + WATER



FLOUR AT 12.0% PROTEIN LEVEL + M-P-B UNFERMENTED



FLOUR AT 12.0% PROTEIN LEVEL + M-P-B FERMENTED

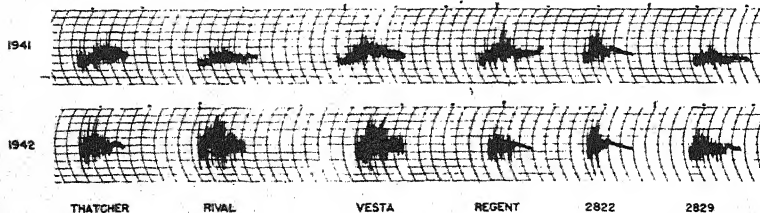


Fig. 2. Comparative mixograms from six hard red spring wheat varieties grown comparably in 1941 and 1942. The lower two lines of curves were obtained by remixing fermented doughs.

development stage and range of stability in both the flour and blend data. These differences seem to be correlated for the corresponding flour and blend mixograms.

Figure 2 shows comparative mixograms obtained from six of the hard red spring wheat varieties grown in 1941 and 1942. Three treatments are represented: flour-water mixes; flour with the malt-phosphate-bromate baking formula unfermented; and flour with the baking formula and 3-hour fermentation. It is apparent that all 1941 curves were

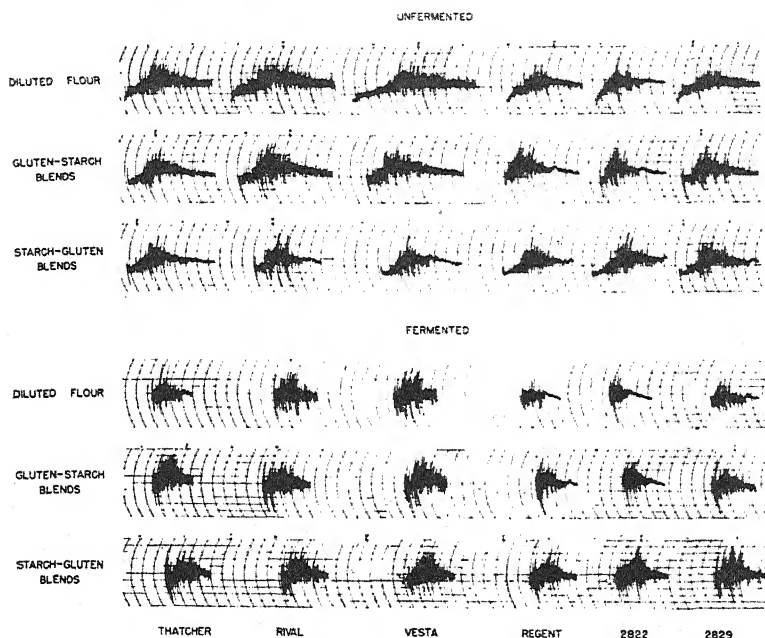


Fig. 3. Mixograms secured from the original flours diluted to 12.0% protein level blended with (a) glutes prepared from the different wheat varieties and a common starch; (b) starches prepared from the wheats and blended with the same hard red spring wheat gluten. Blends made at a protein level of 13.5%.

of a different pattern from the 1942 curves. This difference is very noticeable in the flour-water doughs. Most of the 1941 curves are narrow and rise to a relatively sharp peak. Rival and 2822 are the most noticeable in these respects. The 1942 curves are much broader and less sharply peaked. These characteristic differences are shown by all the methods employed. The baking formula ingredients increase the width of all the curves appreciably. Fermentation, followed by remixing, tended to shorten the curves and reduce the height. Some of the varietal characteristics carry through the fermentation and remixing procedure.

Figure 3 presents a few mixograms taken from comparisons between the original 32 flours and the dried gluten and starch blends.

One set of curves was obtained by mixing the doughs with the malt-phosphate-bromate formula, while the other group was made by remixing after fermentation. Varietal patterns can be discerned in the diluted flour curves and with the blends made with the constant starch substrate and different glutens. Variable starches with the constant gluten, however, do not show these characteristic properties. It is evident that mixing requirements are influenced by the gluten and not by the starch; similar results were obtained by Swanson and Andrews (1943) in studies of hard red winter wheats.

Table III shows an analysis of variance of the data from series one.

TABLE III  
ANALYSIS OF VARIANCE OF THE MIXOGRAM DATA FOR EIGHT WHEAT VARIETIES  
GROWN IN 1942

Source of variation	Degrees of freedom	Variances			
		Dough development stage	Range of dough stability		Curve height
			Total	Post-optimum	
Between varieties	7	20.32 <sup>1</sup>	13.74 <sup>1</sup>	4.67 <sup>1</sup>	0.22 <sup>2</sup>
Between methods	2	98.14 <sup>1</sup>	14.46 <sup>1</sup>	7.89 <sup>1</sup>	11.17 <sup>1</sup>
Between stations	3	3.57 <sup>1</sup>	2.59 <sup>2</sup>	0.72	4.23 <sup>1</sup>
Interactions:					
(Varieties $\times$ methods)	14	1.93 <sup>1</sup>	1.02	0.72 <sup>2</sup>	0.13
(Varieties $\times$ stations)	21	0.84 <sup>2</sup>	0.42	0.24	0.12
(Stations $\times$ methods)	6	1.14 <sup>2</sup>	2.83 <sup>1</sup>	1.57 <sup>1</sup>	0.23
(Varieties $\times$ methods $\times$ stations)	42	0.38	0.81	0.34	0.08
Total	95				

<sup>1</sup> Denotes significance exceeding 1% point.

<sup>2</sup> Denotes significance exceeding 5% point.

The triple interaction, varieties  $\times$  methods  $\times$  stations, has been used as a measure of error. It is apparent that very significant differences exist between wheat varieties in all except one of the measurements; for curve height the difference is significant at the 5% point but not the 1%. Differences in all four properties are extremely significant between methods; Table I shows that this is chiefly because of the effect of fermentation and remixing upon the dough; and this is in accord with *a priori* expectations. The station differences are significant at the 1% point for dough development and curve height, while total range of dough stability is significant at the 5% point only; no significant variations in post-optimum dough stability were found. Significant interactions are shown between varieties and methods for dough development and post-optimum range of stability. For varieties  $\times$  stations the only significant effect is for dough develop-

ment stage, while for stations  $\times$  methods all interactions are significant except for curve height.

Table IV, which deals with series two, shows results somewhat similar to those in Table III. One mixing method, using the malt-phosphate-bromate formula, was employed. Varietal differences in

TABLE IV  
ANALYSIS OF VARIANCE OF THE MIXOGRAM DATA FOR SIX WHEAT VARIETIES GROWN  
IN 1941 AND 1942

Source of variation	Degrees of freedom	Variances		
		Dough development stage	Range of dough stability	Curve height
Between varieties	5	40.07 <sup>1</sup>	12.30 <sup>1</sup>	0.15
Between years	1	0.07	0.90	4.75 <sup>1</sup>
Between stations	3	9.29 <sup>1</sup>	1.35	1.19 <sup>1</sup>
Interactions:				
(Varieties $\times$ years)	5	0.58	0.90	0.20
(Varieties $\times$ stations)	15	1.43	0.43	0.14
(Years $\times$ stations)	3	3.92 <sup>2</sup>	1.56 <sup>2</sup>	0.06
(Varieties $\times$ years $\times$ stations)	15	0.92	0.46	0.16
Total	47			

<sup>1</sup> Denotes significance exceeding 1% point.

<sup>2</sup> Denotes significance exceeding 5% point.

dough development stage and range of stability are very marked, while curve height differences are not significant. Station differences are very significant for dough development and curve height, but not for range of stability, while annual differences are significant only for curve height. For the interactions, varieties  $\times$  stations are not significant throughout; years  $\times$  stations are significant for dough development stage, and range of stability at the 5% point, while curve height is not significant; varieties  $\times$  years interactions are quite insignificant.

The analysis of the gluten-starch blend data for the third series is shown in Table V. The varietal effect on stage of dough development and range of stability is extremely significant, while for curve height there is a significant effect at the 5% point. The effect of method is also very marked for the three properties studied. Station differences are not significant except for curve height which is significant at the 5% point. The interaction of varieties  $\times$  methods is significant at the 5% point for dough development stage and range of stability, and for varieties  $\times$  stations only in dough development. There was no significant interaction between stations and methods.

The data for starch-gluten blends showed results of but minor interest. There were, of course, very significant differences between methods. Varietal and station differences were of little importance,

TABLE V

ANALYSIS OF VARIANCE OF THE MIXOGRAM DATA FOR THE GLUTEN BLENDS WITH A COMMON STARCH

Source of variation	Degrees of freedom	Variances		
		Dough develop- ment stage	Range of dough stability	Curve height
Between varieties	7	6.47 <sup>1</sup>	7.42 <sup>1</sup>	0.22 <sup>2</sup>
Between methods	1	35.10 <sup>1</sup>	18.71 <sup>1</sup>	0.96 <sup>1</sup>
Between stations	3	0.16	0.53	0.31 <sup>2</sup>
Interactions:				
(Varieties × methods)	7	0.31 <sup>2</sup>	1.11 <sup>2</sup>	0.13
(Varieties × stations)	21	0.31 <sup>2</sup>	0.36	0.12
(Stations × methods)	3	0.08	0.31	0.14
(Varieties × methods × sta- tions)	21	0.11	0.35	0.07
Total	63			

<sup>1</sup> Denotes significance exceeding 1% point.<sup>2</sup> Denotes significance exceeding 5% point.

TABLE VI

CORRELATION COEFFICIENTS FOR THE MIXOGRAM DATA

	Variables correlated		$r_{xy}$ <sup>1</sup>
	X	Y	
Dough development stage	Unfermented doughs, flour 12% protein level, cm	Fermented doughs, flour 12% protein level, cm	+0.740
Range of stability	Unfermented doughs, flour 12% protein level, cm	Fermented doughs, flour 12% protein level, cm	+0.560
Dough development stage	Unfermented doughs, gluten- starch blends, cm	Fermented doughs, gluten- starch blends, cm	+0.878
Range of stability	Unfermented doughs, gluten- starch blends, cm	Fermented doughs, gluten- starch blends, cm	+0.709
Dough development stage, un- fermented doughs	Flour 12% protein level, cm	Gluten-starch blends, cm	+0.720
Range of stability, unfer- mented doughs	Flour 12% protein level, cm	Gluten-starch blends, cm	+0.705
Dough development stage, fer- mented doughs	Flour 12% protein level, cm	Gluten-starch blends, cm	+0.856
Range of stability, fermented doughs	Flour 12% protein level, cm	Gluten-starch blends, cm	+0.653
Dough development stage, un- fermented doughs	Flour 12% protein level, cm	Starch-gluten blends, cm	+0.390
Range of stability, unfer- mented doughs	Flour 12% protein level, cm	Starch-gluten blends, cm	+0.355
Dough development stage, fer- mented doughs	Flour 12% protein level, cm	Starch-gluten blends, cm	+0.183
Range of stability, fermented doughs	Flour 12% protein level, cm	Starch-gluten blends, cm	-0.083
Flour, original protein content	Wheat protein content, %	Height of curve, cm	+0.336
Flour, original protein content	Wheat protein content, %	Flour loaf volume, cc	+0.661
Flour doughs, 12% protein level, fermented	Dough development stage, cm	Post-optimum range of sta- bility, cm	+0.794
Flour doughs, 12% protein level, unfermented	Dough development stage, cm	Post-optimum range of sta- bility, cm	+0.698
Flour-water doughs, 12% pro- tein level	Dough development stage, cm	Post-optimum range of sta- bility, cm	+0.471
Gluten-starch blends, unfer- mented	Dough development stage, cm	Post-optimum range of sta- bility, cm	+0.809
Gluten-starch blends, fer- mented	Dough development stage, cm	Post-optimum range of sta- bility, cm	+0.596

<sup>1</sup> Value of  $r_{xy}$  at 5% point 0.352; at 1% point 0.464.

and it is evident that wheat starch had no important role in determining mixogram pattern in these flours.

The more important correlation coefficients which have been calculated for the relations between many of the variables studied are given in Table VI. For dough development stage and range of stability, there is a positive relation between unfermented and fermented doughs with both flours at 12% protein and gluten-starch blends. There are also significant positive relations between the flour doughs and the gluten-starch doughs in each instance, including both unfermented and fermented doughs. In the comparisons between flour and starch-gluten doughs, however, the relations are of little interest. These findings are further evidence that the curve pattern is determined by the gluten. Wheat protein content apparently had little influence on curve height in this series of samples. A positive relation was found between dough development stage and post-optimum range of stability, which would seem to indicate that flours with long mixing requirements also tend to have greater mixing stability.

The data presented indicate that hard red spring wheat varieties may be differentiated to a considerable extent by their mixogram properties. These properties are also significantly affected by the environmental conditions under which the wheats are grown and are inherent in the gluten component. It seems probable that dough mixing requirements are positively correlated with dough stability during mixing.

### Summary

The mixogram properties of a number of hard red spring wheat varieties were examined in respect to (1) the significance of varietal and environmental differences, and (2) the comparative influence of the gluten and starch component of wheat on these differences. Three mixing procedures were also compared respecting their effects on these properties.

Dough development stage, range of dough stability, and curve height were determined by simple measurements taken from the mixogram curves. Significant differences were found among varieties and stations for these properties among eight varieties grown at four locations in 1942. There were highly significant differences between mixing methods. For six varieties grown at four stations for two years, significant differences between years were found only for curve height. In this series, very significant differences were found between varieties for dough development stage and range of stability. Varietal mixing characteristics were reasonably consistent for the two crop



years. In both series, station differences were highly significant for dough development stage and curve height.

Mixograms obtained from blends of gluten and starch showed quite conclusively that varietal differentiation in mixing properties is related to the gluten and not to the starch component.

Fermentation did not change the relations between corresponding mixogram properties. Doughs having a long dough development stage tended to have a greater range of stability.

Several of the wheats examined differed significantly in curve properties from varieties that have been accepted as satisfactory by the milling industry.

The value of the mixograph as an accessory tool in the evaluation of the quality of hard red spring wheat varieties has been demonstrated by the work described.

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## THE USE OF THE MIXOGRAM IN EVALUATING QUALITY IN SOFT WHEAT VARIETIES <sup>1</sup>

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New varieties and selections of soft wheat developed by plant breeders are commonly evaluated by comparing their quality characteristics with those of older varieties whose commercial utility is known. Undoubtedly the best criteria of quality in wheat flours are performance tests in which the flours are actually baked into the products for which they are intended. Unfortunately, evaluation of soft wheat is complicated by the multiplicity of products into which the flours are processed and the lack of standardized baking tests which might serve as adequate indices of quality. For the characterization of soft wheat breeders' samples therefore, it has been necessary to rely largely on chemical, physical, and physiochemical tests. The apparent viscosity of an acidulated flour-water suspension is an example of the type of test which has proved valuable as a quality index.

A recent physical test for wheat flour doughs which has proved valuable for the characterization of wheat varieties, especially in the bread-production field, is the recording dough mixer or mixograph. The instrument, developed by Swanson and Working (1933), was "designed to measure and record automatically the rate of dough development, the duration of resistance against mechanical action, and the rate and extent of increase in mobility of dough as a result of mechanical action." Since these characteristics of dough are a result of inherited traits of wheat varieties, some investigators (Swanson and Working, 1933; Swanson, 1936 and 1939) regard the mixograph as particularly valuable in wheat improvement work in differentiating characteristics due to inheritance. In a study of tests applied to hard red winter flours, Larmour, Working, and Ofelt (1939) concluded that where the protein content is known, the mixograms are most useful in establishing qualitative differences between varieties that may or may not be equal in baking strength.

In connection with a study of baking tests applied to 10 soft wheat varieties, Shellenberger, Hodler, and Nelson (1942) pointed out that mixograms were useful also for indicating differences in the dough characteristics of soft wheat flours.

<sup>1</sup> Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Ohio Agricultural Experiment Station.

<sup>2</sup> Chemist, Assistant Technologist, and Agent, respectively, Federal Soft Wheat Laboratory, Wooster, Ohio. C. E. Bode and H. K. Heizer are now on military furlough with the U. S. Army.

The purpose of the work reported in the present paper was to investigate the mixogram as a measure of quality in the characterization and evaluation of the soft wheat variety samples received at the Federal Soft Wheat Laboratory.

### Material and Method

A National Swanson-Working mixograph, operated in an air-conditioned laboratory maintained at  $80 \pm 1.0^\circ\text{F}$ , was used in this study.

The samples of wheat were milled on an Allis-Chalmers experimental mill. The patent, consisting of break flours and first two reductions, was kept separate from the last two or three reductions until ash determinations could be made on these streams. Enough of

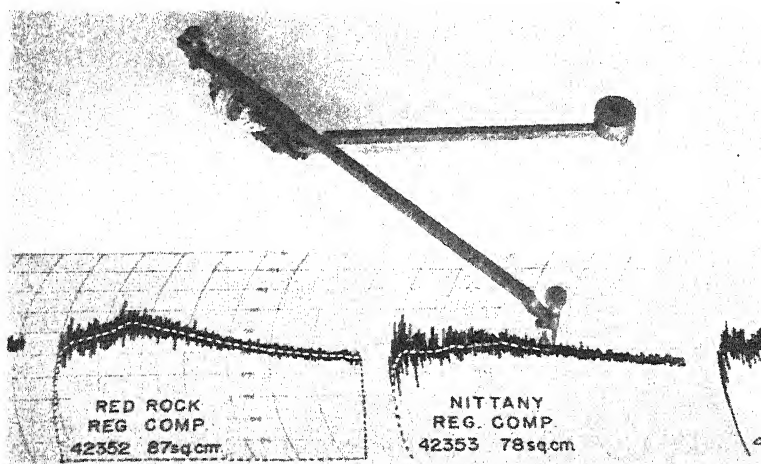


Fig. 1. Measuring mixogram areas with planimeter.

the latter streams was then added to bring the ash content of the flour used for quality tests to about 0.42%. It has been found that on the average this amounts to about 95% of the straight flour.

In developing a procedure suitable for routine samples, two objectives were considered: (1) coverage of the entire range in strength normally found in wheats in the eastern soft wheat region without adjustment of the mixograph, and (2) a single-figure score for expressing general strength or characteristics.

A procedure has been developed which seems to incorporate these features. This procedure includes the use of 35 g of flour (13.5% moisture basis), a spring tension of 8, a mixer head speed of 90 rpm, and a constant absorption of 54%.<sup>3</sup>

<sup>3</sup> This absorption represents an average value for soft wheat flour as determined for bread baking purposes.

In attempting to solve the problem of a single-figure score for expressing the general characteristics of a mixogram, various measurements, including some of those suggested by Johnson and Swanson (1942), were made on a large number of mixograms. The measurement which seemed best to express the gluten strength of each flour sample is the "area under the mixogram."

The measurements reported in this study were made with the Keuffel and Esser Company planimeter shown in Figure 1. The dotted lines in this figure represent the path followed by the planimeter pointer in outlining the "area under the mixogram." The results are read from the planimeter scale in square centimeters. A uniform lapsed time of exactly 7 minutes from start of mixing was included in each area. For simplification, the measurement is referred to in this paper as "mixogram area."

It has been found that although the general characteristics of a mixogram are readily reproducible, there is often a substantial difference in area between replicates even under carefully controlled conditions. On the basis of 1941 and 1942 results, the standard error of a single result has been determined to be 1.745 sq cm. Thus a difference of at least 3.5 sq cm is required for significance between means of duplicates, and 5.0 sq cm for a single mixogram on each sample.

## Results

*Characterization of Varieties by Mixograms.* The use of mixograms in the characterization and evaluation of soft wheat varieties is illustrated by results obtained with the uniform soft winter wheat nursery samples. This series is grown at a number of state experiment stations each year for observations relative to winter hardiness, yield, disease reaction, etc., and includes a number of the most promising newer varieties or selections from the soft wheat region, in addition to many of the older varieties whose commercial utility is known. The results considered in this study involve 29 varieties grown in 1941 at four locations in Ohio (Holgate, Wooster, Columbus, and Miami) and one each in New York, Illinois, Michigan, Indiana, and Kentucky. The grain produced at these locations was composited by variety previous to milling. This series of samples is considered especially suitable for comparing methods of evaluating inherited qualitative differences in gluten characteristics, since differences in protein quantity among these samples are relatively small, and those which do occur are either random differences or are due to an inherited tendency of certain varieties for high or low protein content.

The older varieties which are included to serve as standards of comparison were chosen because they represent the range in gluten

strength from hard red winter to soft white wheats. The ability of the mixograph to differentiate varieties over this range is demonstrated in the mixograms, shown in Table I and Figure 2. Arrangement of

TABLE I

MIXOGRAM AREAS OF VARIETIES GROWN IN EASTERN UNIFORM SOFT WINTER WHEAT NURSERIES; VARIETY SAMPLES COMPOSITED FROM GRAIN GROWN AT NINE LOCATIONS IN 1941

Variety	Mixogram area	Variety	Mixogram area	Variety	Mixogram area
	<i>sq cm</i>		<i>sq cm</i>		<i>sq cm</i>
Kharkof	94	Clarkan	85	Fultz Sel. Hung.	76
Purkof	92	Baldrock	84	Fairfield	74
Brill	92	Fulcaster	83	Gladden	72
		Minhardi	82	Early Premium	71
Mich. Amber	91	Wisc. Ped. No. 2	82	Purdue No. 7	71
Minturki	91	Prairie	80		
Forward	88	Trumbull	80	Yorkwin	67
Red Rock	88	Purdue No. 1	80	Wabash	67
Kawvale	86	Thorne	80	Am. Banner	62
		Nittany	80	Junior No. 6	57
		Purplestraw	79		
		Illinois No. 2	78		

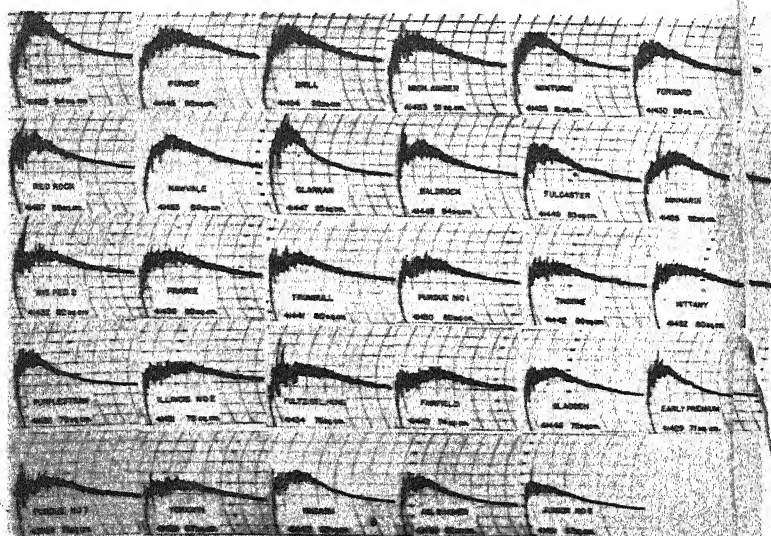


Fig. 2. Mixograms of varieties grown in eastern uniform soft winter wheat nurseries; samples composited from grain grown at nine locations in 1941.

the varieties according to mixogram area is consistent with what is known regarding the relative quality characteristics of the varieties as shown by various physicochemical and baking tests conducted over a period of years.

Of the three varieties having the greater mixogram areas, Kharkof, Brill, and Purkof, the first two are hard red winter varieties, and one parent of Purkof was a hard red winter wheat. The mixograph thus confirms the results of several years' experience with other quality tests in characterizing these three varieties as outstanding among the varieties in this series in gluten strength. Satisfactory commercial utilization of these varieties is limited to bread production.

Ranking the varieties according to mixogram area places in the next group Michigan Amber, Minturki, Forward, Red Rock, and Kawvale. The first mentioned is a selection from a cross between a hard red winter (Turkey) and a soft wheat (Odessa). Other years' results with Minhardi suggest that it also belongs in this group, although the mixogram area of this sample did not so place it. These varieties have been characterized by other quality tests as soft red winter wheats with gluten strength somewhat weaker than the first group, suitable for the most part for family trade flours.

According to mixogram area, Clarkan, Baldrock, Fulcaster, Wisconsin Pedigree No. 2, Prairie, Trumbull, Purdue No. 1, Thorne, Nittany, Purplestraw, and Illinois No. 2 might be included in the next group. Although there is some range in quality exhibited among members of this group, the older varieties are known to produce satisfactory soft wheat flours for such purposes as cakes, cracker sponges, etc. The results of laboratory quality tests agree in characterizing these varieties as medium strength soft red wheats.

From the standpoint of general quality tests there is no clear line of demarcation between the varieties listed in the preceding paragraph and the following: Fultz Sel.  $\times$  Hungarian, Fairfield, Gladden, Early Premium, and Purdue No. 7. Of this group, Fultz Sel.  $\times$  Hungarian, Gladden, and Purdue No. 7 in particular have shown a slight but distinct tendency toward a softer gluten than the other members of the two groups. In this respect the mixogram area and other quality tests are apparently in agreement.

The varieties placed in the weakest group according to mixogram area are, with the exception of Wabash, soft white wheats. Physicochemical and baking tests agree in characterizing Wabash and Yorkwin as being of about equal gluten strength; American Banner and Junior No. 3 as being distinctly softer in quality.

Although in general the varieties are thus arranged in logical groups according to quality reputation and mixogram areas, it should perhaps be pointed out that the smallest area of a variety in one group may vary very little from the largest area of a variety in the following

*Correlation Coefficients between Mixogram Areas and Results of Other Quality Tests.* Additional evidence of the general agreement between mixogram areas and the results of other tests used for quality characterization in this series is afforded by the simple correlation coefficients presented in Table II.

TABLE II  
CORRELATION COEFFICIENTS BETWEEN MIXOGRAM AREA, FLOUR PROTEIN, DOUGHBALL TIME, VISCOSITY VALUES, LOAF VOLUME, AND COOKIE SPREAD FACTOR FOR 29 VARIETIES GROWN IN EASTERN UNIFORM SOFT WHEAT NURSERIES IN 1941

(Value required for significance at 1% point for 29 varieties  $r = \pm 0.470$ )

	Flour protein	Doughball time	Viscosity		Loaf volume M-P-B <sup>1</sup>	Cookie spread factor
			20-g flour	2-g protein		
Mixogram area	+0.659	+0.674	+0.821	+0.693	+0.717	-0.810
Flour protein		+0.379	+0.688	+0.318	+0.642	-0.595
Doughball time			+0.576	+0.583	+0.488	-0.529
Viscosity, 20-g flour				+0.839	+0.736	-0.768
Viscosity, 2-g protein					+0.573	-0.653
Loaf volume, M-P-B <sup>1</sup>						-0.696

<sup>1</sup> Formula B, Bayfield (1941).

Mixogram area gave a high positive correlation with 20-g flour viscosity ( $r = +0.82$ ) and an equally high negative correlation ( $r = -0.81$ ) with cookie spread factor (Hanson, 1943). Mixogram area was correlated with loaf volume to a somewhat lesser degree ( $r = +0.72$ ) although the relationship was highly significant. This was true also of the coefficients between mixogram area and 2-g protein viscosity ( $r = +0.69$ ) and doughball time ( $r = +0.67$ ).

The 20-g flour viscosity determination was, in general, correlated with the other quality tests to about the same degree as was mixogram area. Cookie spread factor and loaf volume were correlated less highly with the results of other tests than was either 20-g flour viscosity or mixogram area.

*Influence of Environment on Quality.* The modifying effects of environmental factors on the quality characteristics of wheat varieties are well recognized. It has been shown by Swanson (1939) that environmental factors, particularly as they influence protein content, cause marked variations in the mixograms of a wheat variety. To the extent to which mixograms of variety samples grown in the eastern soft wheat region are influenced by environmental factors, what is illustrated by mixograms of five well-known commercial varieties as grown at four locations in 1941, shown in Figure 3.

These five varieties represent the range of quality found in the eastern area. Purkof is a semi-hard to hard red winter wheat and is too strong for most purposes except bread. Red Rock when grown under most environmental conditions is regarded as too strong for a

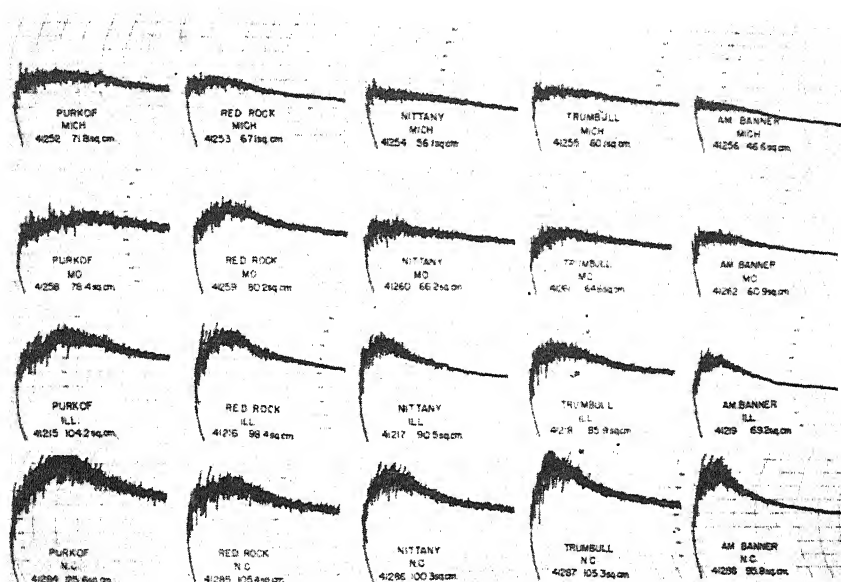


Fig. 3. Effect of environmental factors on mixogram characteristics of five standard varieties grown at four stations in 1941.

satisfactory pastry flour. Nittany and Trumbull are soft red winter wheats of recognized pastry quality. American Banner is a white wheat satisfactory for weaker pastry purposes.

The protein content of the varieties at each station was much the same, although some variation may be noted. The protein contents together with the mixogram areas are given in Table III.

TABLE III

PROTEIN CONTENTS AND MIXOGRAM AREAS OF FIVE WHEAT VARIETIES GROWN AT FOUR LOCATIONS IN THE EASTERN SOFT WHEAT REGION IN 1941

Location	Purkof		Red Rock		Nittany		Trumbull		American Banner		Average	Average
	Protein	Mixogram area	Protein	Mixogram area	Protein	Mixogram area	Protein	Mixogram area	Protein	Mixogram area	Protein	Mixogram area
	%	sq cm	%	sq cm	%	sq cm	%	sq cm	%	sq cm	%	sq cm
East Lansing, Mich.	7.2	72	7.3	67	7.3	56	7.3	60	7.0	47	7.3	62.0
Columbia, Mo.	8.1	78	9.0	80	8.9	66	9.0	65	8.6	61	8.7	70.0
Urbana, Ill.	10.5	104	11.7	98	11.1	91	11.1	86	10.1	69	10.9	89.6
Statesville, N. Car.	14.5	126	13.0	105	13.2	100	13.6	105	12.7	96	13.4	106.4



The immediate attainment of minimum mobility of the dough, characteristic of low-protein soft wheat flours, is well illustrated by the mixograms of the Michigan samples. Only the stronger gluten varieties, Purkof and Red Rock, show some development of gluten with increased mixing time.

When the wheat was grown under environmental conditions (Missouri) resulting in an average flour protein content of 8.7%, the mixograms of even the softer varieties (Nittany, Trumbull, American Banner) began to demonstrate some dough development with mixing. With the samples grown under Illinois conditions, which contained 2.2% more protein on the average than the Missouri samples, there was a further marked increase in mixing time to the point of minimum mobility. These mixograms also show, however, the characteristic rapid narrowing in the weakening slope noticeable in the curves from the lower protein samples.

The mixograms from the North Carolina samples, which contained an average of 13.4% protein, are characterized by no further increase in mixing time to the point of minimum mobility, but by some increase in general width of curve, especially at that point. This level of protein is seldom found in the eastern soft wheat region and represents an average increase of 2.5% over the Illinois samples.

These mixograms demonstrate the effect environment may have on the mixogram characteristics of a variety and serve to emphasize the necessity in comparative evaluation of varieties of using samples grown under uniform environment and with the same relative protein content. It can be noted, however, that at any one location, the five varieties are very well differentiated except for Nittany and Trumbull, which are known to be similar in quality. Although in several instances the differences in mixogram areas do not reach the 5 sq cm required for statistical significance, other characteristics of the mixograms do indicate definite differences in gluten quality. The Purkof and Red Rock samples from Michigan and Missouri are examples of such samples.

### Summary

A satisfactory procedure is described for obtaining mixograms from soft wheat flours for characterizing variety samples over the entire range in flour strength found among samples grown in the eastern soft wheat area.

A single-figure score obtained by measuring the area under the flour mixogram for a mixing time of 7 minutes is suggested as a general expression of strength due to gluten quality and quantity.

In a test of 29 varieties representing a wide range in quality, ranking according to mixogram area was consistent with what is

known regarding the relative quality of the varieties. High correlation coefficients were obtained between mixogram areas and the results of other quality tests, such as 20-g flour viscosity and cookie and bread baking.

Since varietal quality characteristics as expressed in mixograms are modified greatly by environment, comparative evaluation of soft wheat varieties by mixograms can be made only on samples grown under uniform conditions.

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## SIFTED WHEAT MEAL MIXOGRAMS FOR SELECTING SOFT WHEAT VARIETIES

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Quality evaluation of soft wheat samples offers serious difficulties. The flour is used for diverse purposes, and specifications often range within narrow limits, necessitating sensitive tests. Methods have not been developed to the place where they can be interpreted readily in terms of flour usefulness. With new strains, especially in the earlier stages of a breeding and testing program, even greater difficulty has been encountered.

Mixograms seemed to offer possibilities, if modifications of technique could be made. For use with nursery samples, small amounts of grain must suffice, and labor be reduced to a minimum. Milling is almost out of the question. Tests on ground wheat meal appeared to

be the most hopeful. Malloch (1938) used wheat meals in a machine developed by him, and obtained significant correlation ( $r = + 0.88$ ) between the breaking points of meal and flour doughs. More recently Johnson and Swanson (1942) have published a paper dealing with hard wheat meal mixograms. They were able to obtain satisfactory curves, closely similar to the flour curves in their essential characteristics.

The present study was undertaken to explore the possibilities of wheat meal mixograms in the evaluation of soft winter wheat nursery material.

### Experimental

*Development of Technique.* In the spring of 1940, samples of several varieties known to differ in quality characteristics were ground in a Wiley mill, and also in a Jacobson hammer mill and sifted through a 10xx bolting silk. These meals were taken to the Mennel Milling Company Laboratory at Fostoria, Ohio, where mixograms were made using their Swanson-Working recording dough mixer or mixograph. Results were so promising that it was decided to investigate the possibilities further.

A mixograph was purchased by the Ohio station, and exploratory experiments begun on the 1940 crop. Samples were ground in the Wiley mill, Jacobson hammer mill, Labconco burr grinder, Allis-Chalmers experimental mill, and Bantam mikro pulverizer, using various screens and adjustments. The ground wheat was sifted through 64 GG, 6xx, 10xx, and 14xx bolting silks. The mikro pulverizer was considered the most satisfactory grinding device when used with the 0.035-inch herringbone screen. The 10xx bolting silk was selected for sifting.

Sifted meal prepared in this way showed finer particle size distribution than flour from the Allis experimental mill. Some high-ash material was removed in the coarse fractions, but using silks finer than 6xx did not reduce ash appreciably. Protein in the meal also dropped below the wheat samples, as would be expected, and was lowest where the "throughs" were least.

In the fall of 1940, mixograms were made using a head speed of 60 rpm, and a tension setting of 6. This procedure gave poor results; the curves were flat in outline with indistinct maxima in most cases. Where there was much irregularity in the first part of the curve, the true course was often seriously masked. A head speed of 90 rpm was adopted in 1941, with a tension setting of 8, and greatly improved results were obtained.

In general, from the 1940 work it was noted that the finer the meal, the more uniform and clear was the course of the curve; that the silk used to sift the meal was much more important than the screen used in

the micro pulverizer; that low-moisture wheat resulted in high ash and poorer curves; that a 10xx silk was sufficiently fine to separate the meal.

In the fall of 1941, further exploratory tests were made. Wheat was tempered to various levels, and in some cases an additional 2% moisture was added shortly before grinding. The object was to toughen the bran if possible, and reduce the ash in the sifted meal. The attempt was not successful. There appeared to be little advantage in tempering unless samples were below approximately 13% moisture. There was considerable drying of the samples in the mikro pulverizer since it is a very high-speed hammer-type mill with considerable air draft. Accordingly, it was decided to temper to a moisture content of approximately 16% at least 24 hours before grinding.

Violent fluctuations in the first part of the curves were most common on coarse meals, and it was suspected that the hydration of larger particles was slower and less uniform than that of the small particles. Drier meals also gave more trouble, taking longer to settle down to a smooth curve. This result suggested that it might be well to have the meals at as high a moisture as feasible before making the test. Several samples were spread out in a thin layer overnight in a proofing cabinet at high humidity. They took up considerable moisture, but there was no improvement in the curves. Fineness of meal appeared to be the answer to this problem.

The final procedure adopted was as follows: Temper 125 g of wheat to approximately 16% for 24 hours; grind in a Bantam mikro pulverizer, using 0.035-inch herringbone screen; sift 1 minute using No. 120 wire screen (or 10xx silk) and save the "throughs"; determine moisture (5-g samples 30 minutes at 140°C); keep samples at 80°F overnight; weigh out enough meal to contain 30.275 g dry matter (35 g meal at 13.5% moisture); add distilled water (at 80°F) to bring total weight to 55 g and make mixogram immediately; mixograph run 6-7 minutes.

All the work has been carried out in a constant-temperature laboratory maintained at 80°F. Although specific experiments have not been carried out, it has been evident that temperature is critical, and must be maintained within rather narrow limits for satisfactory results.

*Agreement with Flour Mixogram.* Concurrently with this work, Morris, Bode, and Heizer (1944) were developing a technique for use with soft wheat flours. They have found the test one of the best for evaluating such samples. It appeared, therefore, that if sifted meal mixograms agree well with those on flour, the value of the test could be estimated with considerable confidence. To this end, tests were made for two seasons, using the composite uniform soft wheat nursery samples for 1940 and 1941. These included 30 varieties the first year and 29 the second.

Several methods of comparison were used. Figure 1 presents flour and meal mixograms for 10 varieties for the two seasons. Agreement between the two sets is striking, and was equally good for the remaining varieties.

Areas were determined on flour mixograms for 7, 6, 5, and 4 minutes, and on the meal mixograms for 6, 5, and 4 minutes, employing the method described by Morris, Bode, and Heizer (1944). These data were correlated with corresponding data for 20-g flour viscosity. Results are given in Table I.

TABLE I  
CORRELATION BETWEEN 20-g FLOUR VISCOSITY AND MIXOGRAM AREA  
(Mixogram areas for several time intervals)  
(1% level for  $r$ —1940 = + 0.463, for 1941 = + 0.470)

	1940 (30 samples)			1941 (29 samples)		
	7 min	6 min	5 min	7 min	6 min	5 min
Flour	+0.860	+0.843	+0.818	+0.821	+0.810	+0.817
Meal	—	+0.884	+0.858	—	+0.806	+0.794

There is no indication here that mixogram areas are not as satisfactory a measure of quality in meal as in flour.

Correlations for the 7- and 6-minute flour mixogram areas with the 6-minute meal mixogram areas were + 0.923 and + 0.908 respectively for the 1940 samples; and + 0.927 and + 0.919 for the 1941 samples. These are highly satisfactory values.

TABLE II  
MINIMUM, MAXIMUM, AND MEAN MIXOGRAM AREAS ON FLOUR AND MEAL 1940 AND 1941

	Flour mixograms				Meal mixograms	
	7 min		6 min		6 min	
	1940	1941	1940	1941	1940	1941
Minimum	<i>sq cm</i> 56.8	<i>sq cm</i> 57.1	<i>sq cm</i> 53.2	<i>sq cm</i> 51.9	<i>sq cm</i> 47.8	<i>sq cm</i> 48.3
Maximum	102.6	93.8	92.0	82.3	76.4	87.1
Mean	82.6	79.7	75.1	70.5	65.0	67.8

Mean, maximum, and minimum mixogram areas for flour and for meal are presented in Table II. Almost without exception, sifted meal mixograms gave smaller areas than flour mixograms. The range of area values within a series was variable, but was somewhat less for meal mixograms as a rule.

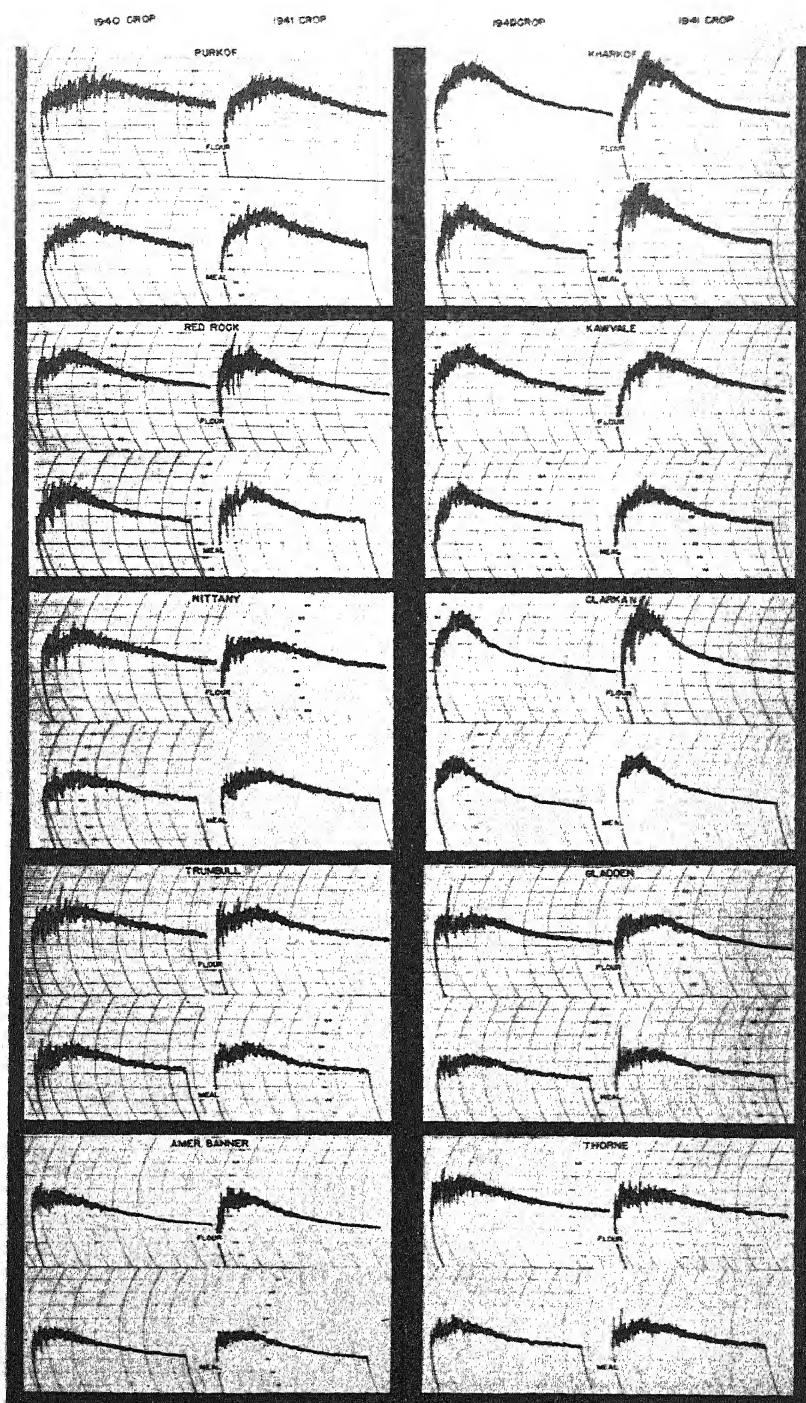


Fig. 1. Flour and meal micrograms from uniform nursery composites 1940 and 1941.

*Value with Wheat Nursery Material.* It is clear that sifted meal mixograms give almost the same picture as flour mixograms. In the opinion of the writer, the sifted meal mixogram gives more information than any other one test that has been used for nursery material. It has been tried now on three crops grown at five locations with satisfactory results. However, mixograms are considerably affected by protein content and probably to some extent by growing conditions, and reference varieties should be included in every test.

Figure 2 shows mixograms from six varieties at five locations in 1941 and 1942. The curve areas are presented in Table III.

TABLE III  
MEAL MIXOGRAM AREAS FOR 1941 AND 1942 NURSERY SAMPLES

Variety	Wooster		S.E. Test		S.W. Test		Holgate	Columbus
	1941	1942	1941	1942	1941	1942	1941	1942
	<i>sq cm</i>	<i>sq cm</i>	<i>sq cm</i>	<i>sq cm</i>	<i>sq cm</i>	<i>sq cm</i>	<i>sq cm</i>	<i>sq cm</i>
Trumbull	65.9	60.3	81.4	62.4	83.4	79.5	57.9	71.0
Thorne	65.4	69.3	75.6	56.2	71.1	65.5	58.7	74.6
Gladden	52.9	62.9	65.6	52.1	74.9	64.6	49.1	63.1
T.N. 1153	85.8	—	93.9	67.0	96.7	92.2	74.2	90.9
T.N. 1159	56.4	67.8	73.4	59.9	78.0	65.2	60.3	68.6
H.S. 269	69.1	—	85.6	69.7	85.3	80.8	62.5	82.6
Average	65.9	—	79.3	61.2	81.6	74.6	60.5	75.1

Single mixograms only were made and error could not be estimated. With flour curves, Morris *et al* (1944) have shown that about 5 sq cm difference was required for significance, and it was not likely less with sifted meal. It can be concluded that for 1941 at Wooster, the Southeast test farm, and Holgate, and for 1942 at Columbus, the ranking of varieties was essentially the same. At the Southwest farm in 1941, agreement was not so good, and all the 1942 data, except those from Columbus, were somewhat erratic.

Lines as strong as T.N. 1153 or H.S. 269 can apparently be picked out in any test. All quality tests had proved them to be unsatisfactory. T.N. 1159, a sister selection of T.N. 1153, was acceptable, but not outstanding.

The 1942 crop in Ohio was definitely abnormal. Leaf rust took a severe toll in some areas, and scab and other diseases were also prevalent. The result was low yields and shrivelled grain. Varieties were not all equally affected, nor were the same varieties most seriously injured at all places. Such a crop is obviously unsatisfactory to use for quality studies and accounts for the results obtained. They are

included here to show that mixograms are no more adversely affected than other tests.

A study of results has shown a correlation ranging around  $+0.80$  between wheat protein and sifted meal mixogram area. Protein is clearly a factor. It is the principal disturbing element in comparing results from two tests. It is sufficiently important that comparison

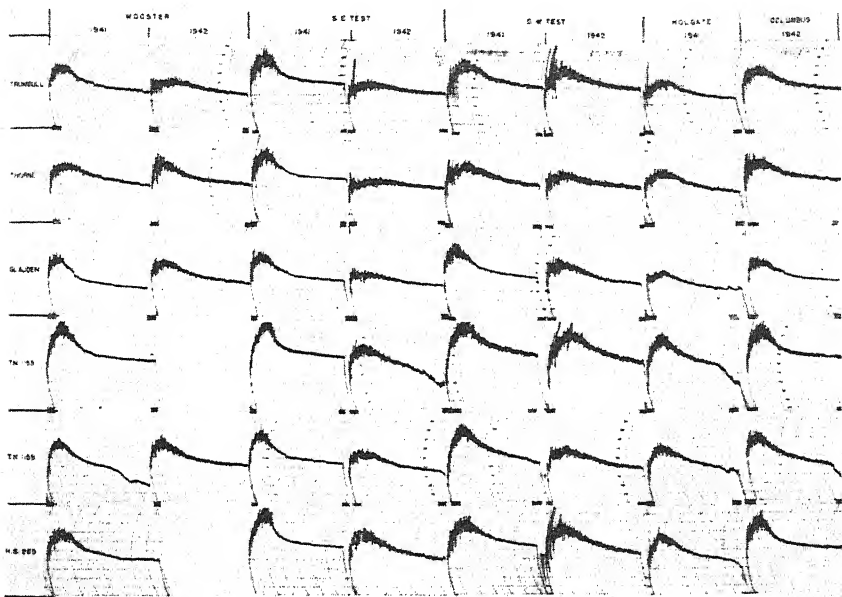


Fig. 2. Meal mixograms for 1941 and 1942 nursery samples.

between strains cannot safely be made unless strains are grown in the same test. On the other hand, when comparing tests, rank of varieties is not seriously affected by high protein at some location or in some season. Experience with the uniform nursery indicates that composites are satisfactory if carefully made.

Johnson and Swanson (1942) noted that the form of mixogram is affected by protein content. Low-protein samples give flatter curves and are more difficult to interpret. The technique outlined has given mixograms which have yielded very useful information in evaluating lines in nursery tests.

*Notes on the Test.* Although characteristics other than area have been studied, none shows as clear-cut association with baking behavior.

The test does not require an excessive amount of labor. Two men can grind and sift 100 samples per day; one operator using two mixing bowls can make seven mixograms per hour. Including one moisture



determination the total labor need not exceed 30 minutes per sample.

The test appears well adapted for use by plant breeders or by mill or elevator operators wishing a reasonably precise estimate of the quality of a lot of wheat.

### Summary

Techniques have been developed for preparing sifted wheat meal from soft wheats, and for making sifted wheat meal mixograms. The meal must be finely ground and the temperature at which the mixograms are made must be carefully controlled. Wheat was tempered to approximately 16°C before grinding. Results reported include data from the Federal uniform soft wheat nursery composites (30 varieties, 2 seasons) and from Ohio wheat breeding nurseries (25 strains, 5 locations, 2 seasons).

The area under the wheat meal mixogram curve (6 minutes), adopted as the quality measure, is highly correlated with flour mixogram area and with other quality tests, such as 20-g flour viscosity.

The test is simple and rapid and can be satisfactorily carried out with 125 g of wheat.

### Acknowledgment

Wheat samples and flour mixograms from the composite uniform nursery were supplied through the courtesy and cooperation of the Federal Soft Wheat Laboratory at Wooster, an agency of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

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## FLOUR BLENDS AND THE QUESTION OF COMPLEMENTARY EFFECTS<sup>1</sup>

KARL F. FINNEY and MARK A. BARMORE<sup>2</sup>

(Presented at the Annual Meeting, May 1941; manuscript received for publication July 1, 1943)

Since an important use of high-protein, good-quality hard red winter wheat flour is for blending with low-protein or poor-quality flours (or a combination of both) to give mixes suitable for bread making, a pertinent question is whether varieties of the former class are accurately evaluated for blending when the flour of each is baked separately.

Flours of certain wheat varieties that appear to be equal in bread-making capacity when baked alone are characterized by considerably different dough-mixing and handling properties. For example, in this laboratory Kharkof and Tenmarq have been shown to be substantially equal in bread-making capacity. On the other hand, curve characteristics as determined by the mixograph suggest that Kharkof is a weaker flour than Tenmarq because of its relatively short developing time and sharp peak. These differences in physical properties suggest that there may be differences in ability to carry low-protein and poor-quality flours even though the baking test indicates equal protein quality when the flours are baked alone. In addition unpublished opinions and studies described in the literature indicate in many cases the presence of complementary effects when flours representing different varieties or classes of wheat are blended in various proportions. In such cases, baking the component flours of a blend separately would not give a reliable measure of the carrying capacity of each.

Harcourt and Purdy (1922) used a lean formula in baking blends of various proportions of Manitoba hard and Ontario soft wheat flours. Their data are reproduced in Figure 1. The solid line is drawn to fit the experimental data, while the broken line represents the theoretical loaf volumes to be expected on the supposition that they are directly proportional to the relative amounts of the flours included in the blend. The data are not in accord with this supposition since those blends containing less than about 40% of Manitoba flour produced smaller loaves than would be expected, while those with 40% or more produced larger loaves than would be expected if there were no "complementary or supplementary effects."

<sup>1</sup> The studies herein reported are a part of the cooperative work carried on by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Agricultural Experiment Stations of the Great Plains Region. Published as contribution number 99 of the Department of Milling Industry, Kansas Agricultural Experiment Station.

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Chapman (1932) states that a 50-50 blend of high-protein hard red spring wheat flour from the Northern Great Plains and a low-protein hard red winter wheat flour from the Southern Great Plains, both of the 1931 crop, was equal in absorption, but had a better color and produced bread with better volume and better texture than that from the hard red spring wheat alone.

Larmour (1931) in studying the relation of protein content and baking quality of hard red spring wheat by means of blends with a club wheat flour increased the proportion of the latter from 40 to 50% be-

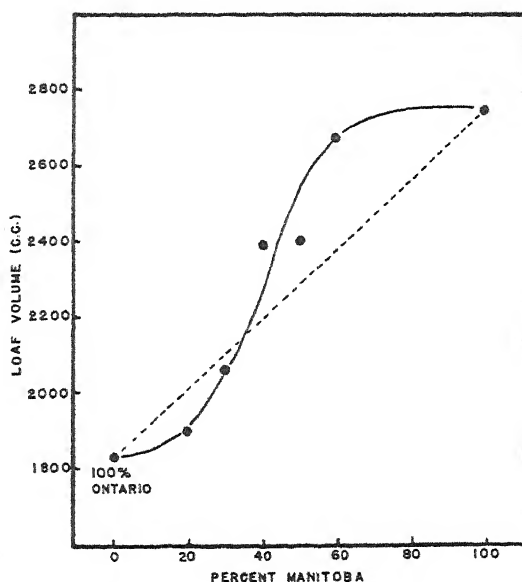


Fig. 1. Loaf volumes for a Manitoba hard, an Ontario soft wheat flour, and their blends (from Harcourt and Purdy, 1922).

cause "in preliminary trials it was observed that some of the higher protein flours showed very little effect of the admixture and it was considered advisable to increase the amount of soft flour sufficiently to insure that even the very strongest flours would show a reduction of volume when compared with the volumes by the bromate formula."

Merritt and Geddes (1943) have reported results of blending experiments showing complementary effects of one flour on another. This, they believe, is due to "the properties of the wheats or flours under study and the baking methods employed."

All of these studies, it will be noted, indicate or imply a complementary effect of one flour on the other. The purpose of this paper is to present certain data bearing on this question.

Complementary is defined according to the dictionary as "serving to fill out and complete, or mutually supplying each other's lack." In the field of cereal chemistry this term has been used in this sense to indicate effects different from the expected average of the components. Flours showing complementary effects in a blend would yield in that blend baking results different than the proportionate average of those produced by the components when baked individually.

### Materials and Methods

In the studies reported herein, the flour from each of five varieties of hard red winter wheats, Kharkof, Cheyenne, Nebred, Oro × Tenmarq C.I.<sup>3</sup> 11672, Chiefkan, and one variety of spring wheat, Thatcher, was blended with each of three other flours in 25-75, 50-50, and 75-25% blends. The five winter varieties are known to differ greatly in flour properties and baking quality characteristics. The three flours blended with them included (1) a low-protein good-quality soft winter composite (38535) furnished by the Mennel Milling Company, Toledo, Ohio, (2) a medium-protein very-poor-quality hard winter (38536) milled from Chiefkan, and (3) a medium-protein good-quality hard winter (39067) milled from a composite lot of grain representing approximately 7000 cars of wheat collected at the terminal markets under the supervision of the Grain Products Branch of the Food Distribution Administration, U. S. Department of Agriculture. The winter wheats

TABLE I  
PROTEIN CONTENT, OPTIMUM MIXING TIME, AND BROMATE REQUIREMENT FOR EACH OF NINE FLOURS

No.	Variety	Flour protein <sup>1</sup>	Optimum mixing time	KBrO <sub>3</sub> requirement
		%	min.	mg
39901	Kharkof	15.7	1½	5 to 6
39906	Cheyenne	15.3	4½	3
39907	Nebred	15.7	3½	3
39909	Oro × Tenmarq	15.7	3½	4
39912	Chiefkan	15.2	1½	6
39722	Thatcher	14.6	3½	2 to 3
39067	Commercial composite	13.0	2½	5
38535	Soft red winter	8.2	2½	1 to 2
38536	Chiefkan	12.2	1½	4

<sup>1</sup> 13.5% moisture basis.

for these studies were grown in experimental plots in the Central and Southern Great Plains in 1939 and were milled on a Buhler mill. The flour from the spring wheat, Thatcher, generally recognized as of excellent quality for bread making, was milled from a composite lot of

<sup>3</sup> C.I. refers to accession number of the Division of Cereal Crops and Diseases.

four samples of grain grown in the Northern Great Plains and supplied by the Northwest Crop Improvement Association. The protein content, optimum mixing time, and bromate requirements for each of the nine flours are recorded in Table I. The laboratory numbers are also given for identification purposes later.

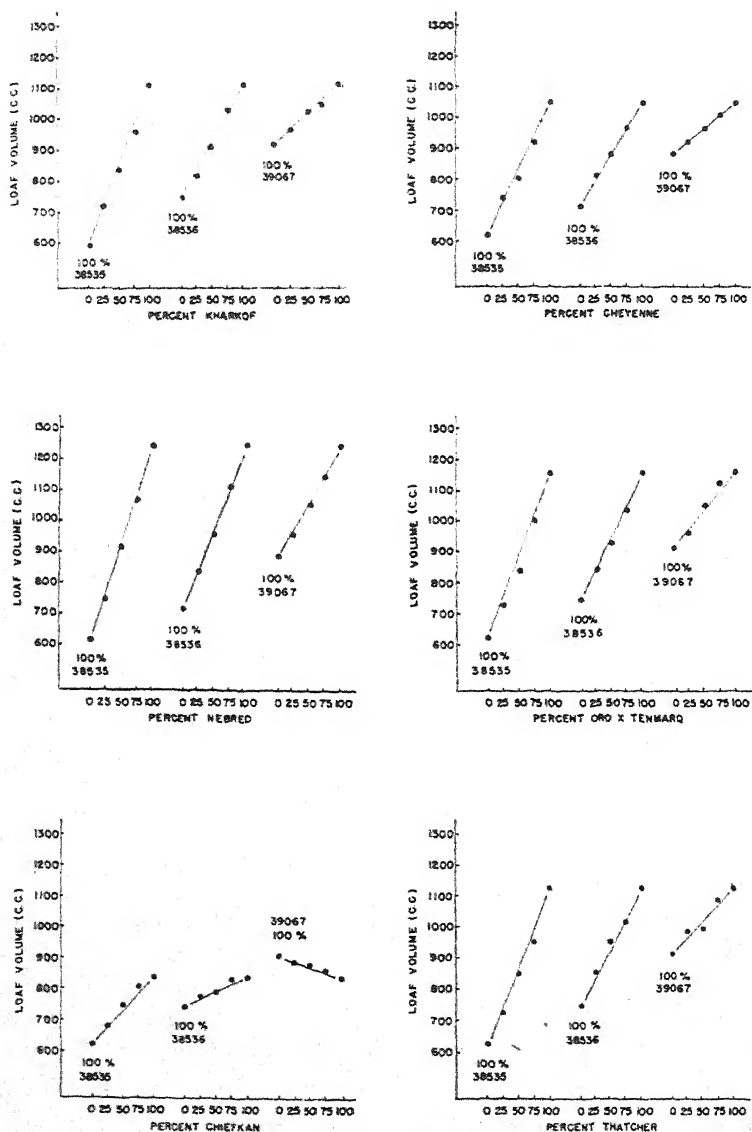


Fig. 2. Flours from one hard spring and five hard winter varieties in blends with a good-quality 8.2% protein S.R.W. (38535), a poor-quality 12.2% protein H.R.W. (38536), and a good-quality 13.0% protein commercial composite (39067).

Each original flour and blend was baked on each of three days using the A.A.C.C. straight-dough baking test procedure in conjunction with optimum mixing time (Finney and Barmore, 1941) and a baking formula (Finney and Barmore, 1939 and 1941a) containing the following ingredients per loaf: 100 g flour, water as needed, 6 g sugar,  $1\frac{1}{2}$  g salt, 3 g shortening, 2 g yeast, 4 g milk solids,  $\frac{1}{4}$  g 120° L malt syrup, and 3 to 6 mg  $\text{KBrO}_3$ , depending on the variety. The amount of  $\text{KBrO}_3$  used in the blends (0 to 100%) was 3 mg for Nebred and Cheyenne, 4 mg for Oro  $\times$  Tenmarq, Chiefkan, and Thatcher, and 6 mg for Kharkof. The absorptions and mixing times used for the blends were calculated, assuming each to be directly proportional to the amounts of the different flours present. These calculated properties were found to coincide with the observed experimental values, thus indicating the assumption to be correct.

### Discussion

The loaf volumes for the various flour blends and for each flour when baked alone are shown in Figure 2. The percentage of each flour in the blend is given on the abscissa and loaf volume in cc on the ordinate. The lines indicate the expected loaf volumes on the basis of no complementary effects.

In Figure 3 the experimental loaf volumes of all blends are plotted against the volumes calculated on the assumption of no complementary effects. The correlation coefficient for the 54 lots was found to be +0.988.

The data appear to provide no evidence of complementary effects. Not only is the correlation coefficient extremely high, but also there is not a single case in which the difference between actual and calculated volumes is greater than would be expected as a result of random errors.

The crumb grain scores and the crumb color scores of Oro  $\times$  Tenmarq and the blends of this variety with the three blending flours are presented graphically in Figure 4. The results here shown are typical of the other varieties. It will be noted that the crumb grain scores given at the top of the figure suggest marked complementary effects, since with one exception the blends have higher scores than either flour alone. This result, however, is believed to be due to the relation between protein content and crumb grain scores. The maximum score in every case for all varieties as well as for the Oro  $\times$  Tenmarq in Figure 4 was obtained for blends containing about 14% protein, which seems to represent about the optimum protein-starch ratio for the best grain scores. Blending with a lower-protein flour appears simply to reduce the protein content of the blend to a value such that the factors of high volume and high protein-starch ratio do

not function in the production of more open grains and lower scores. In this sense there is a complementary effect. It would appear, therefore, that if the tendency of high-protein flours to produce open grains and low scores is overlooked or ignored, varieties represented by high-protein flour could be erroneously evaluated, as compared with their value in blends with low-protein flour. If this relation is taken into

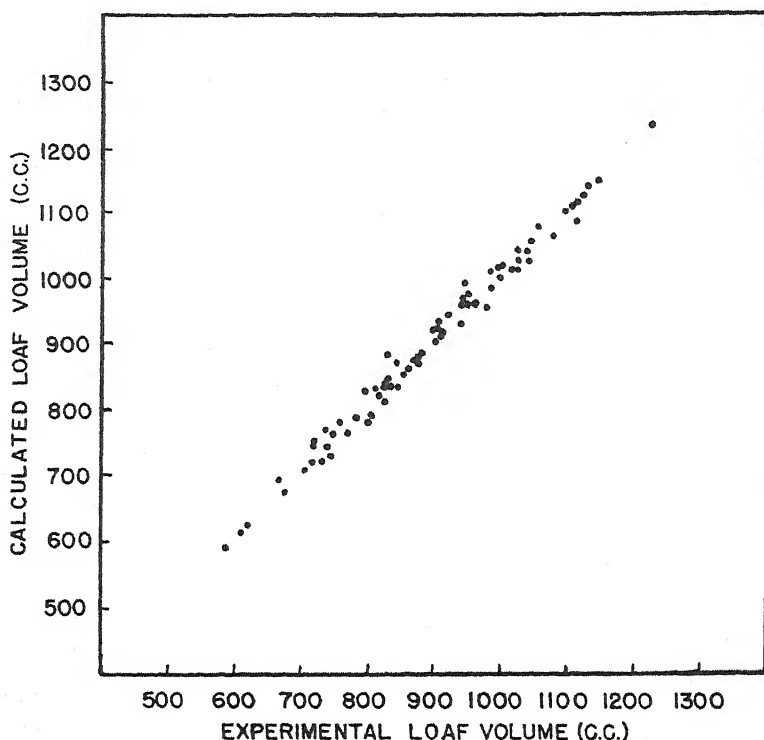


Fig. 3. Calculated and experimental loaf volumes of all flours and their blends.

account, however, there should be no confusion in the comparative evaluation of the crumb grains for different varieties.

The crumb color data for Oro  $\times$  Tenmarq alone and with the three blending flours shown in the lower half of Figure 4 indicate that the crumb color of a blend is a function of the crumb colors of the flours making up the blend. Here, again, the data for Oro  $\times$  Tenmarq are typical of those for the other varieties.

It seems probable that the excellent agreement between calculated and experimental loaf volumes of the blends, and the absence of complementary effects, except with respect to crumb grain, is due to the baking methods used in this laboratory. The features believed to be

important are the use of absorptions, mixing times, and bromate levels appropriate for each variety and blend, the use of milk solids to buffer any deleterious effects of overbromating, and sufficient sugar, malt,

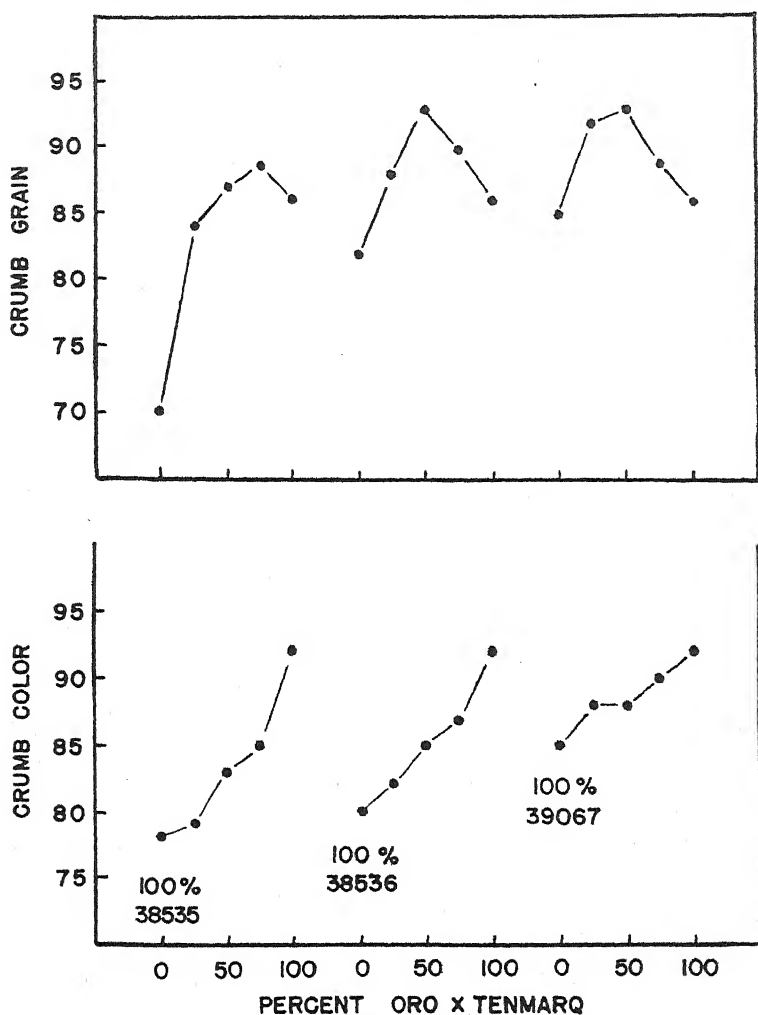


Fig. 4. Crumb grain and crumb color scores for the bread baked from Oro X Tenmarq, three blending flours, and their blends.

and yeast to insure an adequate amount and rate of gas production necessary for proper dough development. This procedure, it is believed, permits a complete or nearly complete expression of the potentialities of each variety and appears more nearly to measure "protein quality" than any other baking method used in this labora-



tory. When it is used, there appears to be left but little if any room for complementary effects on loaf volume. As noted above, the tendency of high-protein flours to produce undesirable crumb grain must be taken into account if erroneous conclusions are to be avoided. This, of course, applies to blends as well as to nonblends except that in commercial practice high-protein blends are not likely to be encountered.

Therefore, it is logical to conclude that hard wheat varieties having approximately equal protein content and equal loaf volume producing ability, in general, do not vary in their abilities to carry weaker flours. The relative strength or weakness of the various flours from the standpoint of either quality or quantity of protein is measured by loaf volume obtained by the baking test previously described. The relative ability of different flours to carry weaker flours is accurately indicated by their loaf volumes when baked alone.

TABLE II

AMOUNT OF SOFT RED WINTER WHEAT FLOUR THAT EACH OF SEVERAL VARIETIES OF HARD WHEAT FLOUR WILL CARRY AND PRODUCE A LOAF VOLUME OF 840 CC

Variety	As received			at 13% protein	
	Protein	Loaf vol.	S.R.W. carried	Loaf vol. <sup>1</sup>	S.R.W. carried
Nebred	15.7	1240	64	1024	45
Oro X Tenmarq	15.7	1155	60	966	37
Thatcher	14.6	1123	57	1003	43
Kharkof	15.7	1114	53	939	29
Cheyenne	15.3	1049	48	911	24
Chiefkan	15.2	840	0	763	— <sup>2</sup>

<sup>1</sup> Calculated on the assumption of linear relationships between protein content and loaf volume and a change in loaf volume for each percent protein of 80 cc for Nebred, 75 cc for Thatcher, 70 cc for Oro X Tenmarq, 65 cc for Kharkof, 60 cc for Cheyenne, and 35 cc for Chiefkan.

<sup>2</sup> Chiefkan alone at 13.0% protein would be expected to produce a loaf volume of only 763 cc.

*Carrying Ability of Varieties.* An interesting and instructive way to express the protein quality of hard wheat varieties is in terms of the percentage of a low-protein, or low-quality flour that can be blended with it and still produce a satisfactory loaf of bread. Table II shows the protein content of the flours, the loaf volumes secured in the baking tests, the loaf volumes that would be expected if each variety had the same protein content (13.0%), and the proportion of a soft red winter wheat flour that could be blended with each variety and produce a loaf with a volume of 840 cc, the latter being the loaf volume of Chiefkan, the poorest of the hard wheats. The soft red winter wheat flour is the same as that in Table I with 8.2% protein. The expected loaf volumes at 13.0% protein are calculated on the basis of the slopes of the regression lines previously determined in this laboratory for each variety.

The calculations show that Nebred can carry 64% of the 8.2%

protein soft red winter flour; Oro X Tenmarq 60%; Thatcher 57%; and finally Chiefkan, which can carry none.

On an equal protein basis of 13.0%, Nebred and Thatcher are more nearly equal and would be expected to carry 45% and 43%, respectively, of the soft red winter. Oro X Tenmarq is somewhat lower in carrying ability at 37%, Kharkof 29%, Cheyenne 24%, while Chiefkan could carry none. In fact the loaf baked from *all Chiefkan flour* containing 13.0% protein would have an expected loaf volume of only 763 cc, which is considerably less than the desired 840 cc.

### Summary

Flours of one hard spring and five hard winter varieties having widely different properties, and varying in protein content from 14.6 to 15.7%, were blended in various proportions with three other flours characterized as follows: (1) a low-protein, good-quality soft red winter wheat flour; (2) a medium-protein, poor-quality hard red winter flour; (3) a medium-protein, good-quality, hard red winter flour. The individual flours and their blends were baked into bread and the results compared.

The loaf volumes and bread color scores provided no evidence of complementary effects previously reported when flours of widely different properties have been blended. Both were found to be directly proportional to the amount of each flour in the blend.

The grain scores likewise indicate no complementary effects provided allowance is made for the type of grain normally found in bread from high-protein flours. The grain in such bread is usually uneven and open, but if from flour of good quality, this characteristic disappears when blended with lower-protein flours in accordance with commercial practice, and the close, lacy, elongated cell structure characteristic of good-quality bread takes its place.

It appears that complementary effects are largely a function of the methods used in the baking test. In the work reported herein, the absorptions, mixing times, bromate levels, etc. were such that the potentialities of each variety were probably sufficiently expressed. Under such conditions there is probably less opportunity for complementary effects than would otherwise be the case.

It appears, therefore, that a flour's carrying power is a function of protein quality and quantity, and can be measured by the loaf volume obtained with the rich, highly bromated, milk-containing formula.

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## THE INHIBITORY EFFECT OF A PROTAMINE FROM WHEAT FLOUR ON THE FERMENTATION OF WHEAT MASHES <sup>1</sup>

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Experiments already described by Stuart and Harris (1942) have demonstrated that a protamine<sup>2</sup> derived from wheat flour not only prevents in very small concentrations the multiplication of *S. cerevisiae*, but in greater concentrations kills this yeast. More recent experience has shown that the protein also inhibits alcoholic fermentation; *i.e.* that the yeast cells affected by it no longer ferment sugar well, if indeed at all. Although this protein exists in wheat only in combination with other substances, among them lipoids, it was a matter of some wonder that its presence did not always interfere seriously with the yields of alcohol produced by yeast fermentation. There is reason to believe that in some cases low yields of alcohol have been encountered because of this substance. But there is also abundant evidence that such is not always the case; in fact it is the exception rather than the rule.

The following study shows that inhibition of wheat fermentation by this toxic protein is a distinct likelihood, particularly when "granular flour" rather than whole wheat is used. However, some of the present methods of mashing and fermentation (perhaps accidentally) eliminate this danger. This report is therefore meant to show something of the underlying mechanism involved in the destruction of the toxic

<sup>1</sup> Enzyme Research Laboratory Contribution No. 84. This work done in part under Special Research Funds, SRF-2-9.

<sup>2</sup> The name purothionin has been proposed for this substance because of its very high content of sulfur by Balls, Hale, and Harris (1942a).

protein during ordinary fermentations, whereby its inhibitory effect is largely prevented. The experiments have been made with crystalline purothionin hydrochloride, entirely on a laboratory scale, because only laboratory quantities of the pure material were available. The principles appear to be applicable to materials in any quantity.

Purothionin has been isolated as a crystalline protamine hydrochloride from wheat flour (Balls, Hale, and Harris, 1942). It is remarkable for its high content of cystine and arginine, its power to function as an oxidation-reduction system, its basicity, and its tendency to undergo an apparent "alkaline" denaturation even at slightly acid pH levels. The toxicity of this substance appears to be due to the arrangement of the amino acids in the molecule and not to the presence of a constituent part toxic in itself. It follows that any method of breaking down the molecular arrangement destroys the toxicity. This has been previously demonstrated on small animals injected with purothionin exposed to the action of crystalline trypsin, chymotrypsin, chymopapain, and crude papain.

In the preparation and fermentation of wheat mashes two steps occur that have been found to destroy the molecular structure on which the toxicity depends: heating, and proteolysis by enzymes in the malt and possibly in the yeast. Toxicity also has been destroyed by increasing the yeast inoculum and by decreasing the acidity of the fermentation to a pH over 5. These schemes are probably just ways of increasing proteolysis, by liberating proteinase from dead yeast cells, or by producing a pH more favorable to the action of the malt enzymes. It may be concluded that the toxic protein in wheat may be removed by cooking the mash thoroughly or by the use of an actively proteolytic malt.

### Procedure

*Cooking:* One hundred ml of water was added to 25 g of meal or flour in a 300-ml Erlenmeyer flask and cooked at various temperatures. For cooking at 80° a constant temperature bath was used and at 100° an Arnold oven was used. The cooking time was 1 hour, unless otherwise specified. Pressure cooking was done in a laboratory autoclave at 15 lb pressure per square inch.

*Mashing:* After cooling the cooked mash to 55°, 100 ml of malt slurry (2.5 g of ground malt soaked 1 hour at room temperature with 100 ml water) was added and the mixture kept at 55° for 1 to 1½ hours.

*Fermentation:* The mash was cooled to 32° and inoculated with distillers' yeast. The yeast cells were grown in wort broth for two or three days until the cell count was between 30 and 50 million cells per ml. A quantity of broth containing the desired number of cells

was centrifuged and the cells were washed into the mash with about 2 ml of water.

*Alcohol Determination:* The alcohol was determined as usual by distilling off about one-half of the mash, diluting the distillate to a definite volume and determining the specific gravity at 25°.

Progress of the fermentation was followed by determining the loss in weight owing to evolution of CO<sub>2</sub>. The flasks were provided with CaCl<sub>2</sub> tubes to prevent loss of water or alcohol in the course of the fermentation. The results were not corrected for CO<sub>2</sub> remaining dissolved in the mash nor for that which replaced air above the surface of the liquid. They are therefore approximate.

## Results

The inhibitory effect of the wheat protamine on alcoholic fermentation was found to depend on pH, the size of the yeast inoculum, and whether or not the inhibitory substance had previously been exposed to proteolytic enzymes. These effects were shown by experiments in which crystalline purothionin hydrochloride was added to sterilized (10° Bal.) malt wort. In such wort with an initial pH of 5.28 and an initial yeast count of  $28 \times 10^6$  cells per ml, the concentration of purothionin critical for inhibiting fermentation (shown by gas formation within 48 hours at 32°) was between 30  $\mu$ g and 40  $\mu$ g per ml. With 50  $\mu$ g per ml and the same yeast inoculum, gas formation was nil or slow at pH levels below about 4.7, but normal thereabove. At its normal pH of 5.28, the wort fermented vigorously when inoculated with only  $2.8 \times 10^6$  yeast cells per ml, without any purothionin, but the presence of 50  $\mu$ g per ml of the protamine prevented any fermentation with 10, 20, 30, or 40 times this quantity of yeast. However, vigorous fermentation occurred in the presence of the same amount of purothionin when  $280 \times 10^6$  cells per ml (100 times the first inoculum) was used.

Pretreatment of wort containing 1 mg purothionin per ml (or about 30 times the critical concentration) with an equal weight of crystalline trypsin for about 3 hours at 35° (pH 5.0) permitted a vigorous fermentation after an inoculum of  $26 \times 10^6$  cells per ml. The trypsin itself was not inhibitory.

Similar prevention of the inhibitory effect of purothionin was observed when it was added to miniature mashes of whole corn meal that had been malted. The concentration of inhibitor used was high (2 mg per ml of "fermenter" liquid), but it appeared to be completely destroyed by the malt proteinase.

Further experiments were then confined to miniature mashes of wheat, to which no additions of purothionin were made. These behaved as though the wheat contained the same inhibitory substance, capable of destruction by proteolysis. Thus whole wheat meal (wherein the bran contained considerable proteinase) fermented very well, even when cooked at only 80°. On the other hand, mashes of "granular flour" (an 85% extraction) fermented very poorly unless heavily inoculated with yeast or else thoroughly cooked under pressure. An experiment is shown with whole wheat in Table I and with granular flour in Table II.

TABLE I  
FERMENTATION OF WHOLE WHEAT MEAL (KANSAS HARD) AFTER  
COOKING AT VARIOUS TEMPERATURES

Cooked for 1 hr at ° C	Loss of CO <sub>2</sub> after			Alcohol yield after 49 hr (ml/100 g wheat)
	6.5 hr	23 hr	49 hr	
	g	g	g	
80	0.2	5.5	6.0	31.6
80	0.1	5.6	6.1	31.6
100	0.2	5.3	5.7	30.6
100	0.1	5.6	6.1	32.6
Autoclaved	0.3	5.4	6.0	32.2
Autoclaved	0.2	5.5	5.7	31.0

Yeast inoculum was  $34 \times 10^6$  cells per ml.

TABLE II  
FERMENTATION OF GRANULAR FLOUR (MINN. HARD WHEAT)

Cooked for 1 hr at ° C	Yeast inoculum	Fermentation (time in hours)	Alcohol yield (ml/100 g wheat)
Experiment I			
1. 80	$26 \times 10^6$	75	2.9
2. 100	$26 \times 10^6$	75	30.0
3. Autoclaved	$26 \times 10^6$	75	38.3
Experiment II <sup>1</sup>			
1. 80	$37 \times 10^6$	42	23.0
2. 100	$37 \times 10^6$	42	39.0
3. Autoclaved	$37 \times 10^6$	42	37.0
Experiment III			
1. 80	$4.6 \times 10^6$	43	6.1
2. 80	$9.2 \times 10^6$	43	9.2
3. 80	$13.8 \times 10^6$	43	9.2
4. 80	$18.4 \times 10^6$	43	14.7
5. 80	$37.0 \times 10^6$	42	23.0

<sup>1</sup> Experiment II was a duplicate of Experiment I but a more actively growing yeast was used.

The existence of the inhibitor in wheat was most clearly demonstrated by adding dextrose and yeast, but no malt, to uncooked granular flour. The presence of the uncooked flour prevented the fermentation of the dextrose. If the flour was cooked at 80°, fermentation was poor. Cooking the flour at 100° permitted about half the dextrose to be fermented (46 hours), while autoclaving the flour allowed practically complete fermentation (Table III). However, when uncooked flour was first treated with crystalline trypsin and then mixed with yeast and dextrose under conditions comparable to the former experiment, fermentation of the sugar was rapid (Table IV).

TABLE III  
FERMENTATION OF GRANULAR WHEAT FLOUR (MD. SOFT WHEAT)  
TO WHICH DEXTROSE<sup>1</sup> BUT NO MALT WAS ADDED

Cooked for 1 hr at ° C	Loss of CO <sub>2</sub> after		
	22 hr	28 hr	46 hr
	g	g	g
Not cooked	0.6	0.8	0.8
80	0.9	1.2	1.4
100	3.0	3.1	4.1
Autoclaved	6.4	7.7	9.2

<sup>1</sup> Twenty g of dextrose (about 10%) was added to each flask; the yeast concentration was  $34 \times 10^6$  cells per ml mash.

TABLE IV  
FERMENTATION OF DEXTROSE MIXED WITH UNMALTED, UNCOOKED GRANULAR  
FLOUR THAT HAD BEEN EXPOSED TO CRYSTALLINE TRYPSIN

	Loss of CO <sub>2</sub> after		
	1.75 hr	5 hr	44 hr
	g	g	g
1. Control	0	0	0
2. Trypsin-digest	0.7	3.6	9.1

To 25 g granular flour (soft winter wheat) 200 ml water and 200 mg wet crystalline trypsin filter cake (actually about 50 mg trypsin) were added. The pH was adjusted to 8.0. 20 g of dextrose was added and the mash kept at 37° for about 16 hours. The mash then was cooled to 32°, pH adjusted to 5.0 and inoculated with centrifuged yeast cells to make a concentration of  $34 \times 10^6$  cells/ml. The control was treated in the same way except that no trypsin was added.

### Summary

Evidence of a fermentation inhibitor in wheat has been presented. In the fermentation of whole wheat meal, this substance appears to be either neutralized or in some manner destroyed by other substances that must occur in the bran. In the fermentation of granular flour, the toxic substance was found to be present and active. It may be destroyed by proteolysis or by pressure cooking; partly destroyed by heating to 100° for 1 hour, but it is very little affected by a cooking temperature of 80° for 1 hour.

### Acknowledgment

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## BOOK REVIEW

**Physical Properties of Dough.** By C. O. Swanson. 258 pp. Burgess Publishing Company, Minneapolis, Minnesota. 1943. Price \$2.25.

In the foreword of this book Professor Swanson states that it has been his aim to express ideas in such terms that those who have not had courses in physical and colloid chemistry may nevertheless use the book with profit. It is evident that this policy has been followed in all sections of the document, and the fundamental equations, and certain of the precise definitions of the fundamental physical properties of matter either are not included in the discussion, or are considered very briefly and in general terms.

The book opens with a discussion of certain colloidal phenomena represented in bread and dough. Then follows a consideration of starch and proteins as dough constituents. The author then returns to colloidal and related physicochemical properties, including surfaces and particles, aggregation of particles, adsorption, surface films, free and bound water, adhesion, and interfacial tension. Particular stress is laid upon the state of water in dough, and the formation and properties of dough.

The physical states of matter are mentioned in the opening paragraphs of Chapter VII, followed by a discussion of viscosity, plasticity, and elasticity. In the last two chapters these properties reappear to some degree in the author's treatment of his primary thesis, the physical tests of quality. Absorption or flour-water ratios in doughs and the use of various plastometers and penetrometers, elasticity of dough and the degree to which it is altered by various manipulations, the extensimeter, and recording dough mixers receive particular attention. Swanson refers briefly to the Hogarth, Hankoczy ("farinograph"), and Malloch recording dough mixers, but gives particular attention to the action of the Hobart-Swanson mixer, and the application of the planetary action in the mixer to the recording dough mixer which he developed. Characteristics of the graphs or "mixograms" plotted by the recording mechanism are described at some length, based upon type graphs depicted in Figure 15. The influence of protein content upon mixing behavior is stressed, and demonstrated with a collection of mixograms. Also the effects of various treatments and of certain dough ingredients upon mixing behavior are discussed.

The author agrees that the correlation between mixogram characteristics and qualities of the baked loaf has not been established abundantly. He submits further that the greatest usefulness of the mixogram lies in the information that it may furnish to supplement the data resulting from baking tests. Mixing requirements and mixing tolerance are listed among these supplementary findings. He also proposes that when a flour type is established as possessing poor baking qualities, its mixogram may be taken as a pattern of such poor qualities and it may be assumed that other flours exhibiting similar mixing characteristics are likewise inferior in baking qualities. The specifications of such mixograms are not recorded with great definiteness however.



Some omissions should be noted in Professor Swanson's treatment of physical properties of doughs. Thus there is no discussion of adhesiveness or "stickiness," or of work hardening. The latter appears to the reviewer to be of primary significance in a comprehensive survey of this subject.

Legends for certain of the figures in the book are brief and incomplete and their usefulness could be enhanced by expanding them, notably in the instance of the legends which accompany the mixograms. Also there are numerous typographical errors, but most of them are of minor consequence.

In general, Professor Swanson has provided a useful elementary discussion of the subjects included under the title of this book, and particularly for those that are not concerned with a more fundamental approach.

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## EFFECTS ON FLOUR DOUGHS OF EXTRACTS FROM UNFERMENTED AND FERMENTED WHEAT GERM

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The effects on doughs of oxidizing substances, of reducing substances, and of proteases activated by —SH groups have for several years past attracted the attention of cereal chemists, and numerous investigations in this field have been carried out.

Shen and Geddes (1942) gave a comprehensive review of the literature dealing with these problems up to the second half of 1941. Their paper and other recent papers on the subject under discussion will be reviewed in the following paragraphs.

Shen and Geddes (1942) studied the effect of length of extraction and time of fermentation on bromated and nonbromated doughs and on doughs in which yeast activity was inhibited by octylalcohol. (This was added to prevent the yeast from using up the amino acids produced as a result of proteolysis.) They determined the amino nitrogen and reducing matter content of the doughs with a view to correlating them with the baking performance of the flours, and they reported that the fermentation time and/or bromate required to produce a satisfactory dough and loaf increased as the flours contained more of reducing matter and protease. On the other hand, however, they observed that the reducing matter content of the doughs increased with longer fermentation times, while it decreased when bromate was added. The amino nitrogen content of the nonfermenting dough increased with length of dough time.

Hullett (1940), investigating the effects on doughs of prefermented wheat germ suspensions, found that the nitroprusside reaction of a fermenting germ suspension becomes negative after a certain time, and he expressed the view that the elimination of glutathione may be an important part of the ordinary dough ripening process.

Hullett and Stern (1941), working on the same subject, showed that the elimination of glutathione from a fermenting germ suspension is

connected with the fermenting activity of the yeast on the one hand and with an enzyme mechanism present in the raw germ on the other. They reported that the disappearance of the nitroprusside reaction is not due to the oxidation of the —SH group of glutathione to the —S—S— form but to a more far-reaching change, and they showed that germ fermentation caused the nitroprusside reaction to become negative in the yeast as well as in the germ.

Smith and Geddes (1942) investigated the effect of 5% and 10% additions of germ, untreated and pretreated, on a highly refined untreated second middlings flour and on mixtures of this flour with 10%, 15%, or 20% of added wheat starch. The germ suspensions were pretreated by allowing them to stand for varying times with or without addition of bromate, or yeast, or yeast plus bromate. They found that the injurious effect of untreated wheat germ decreased as dough fermentation was extended from 1.5 to 4.5 hours. Addition of potassium bromate to the dough caused marked improvements of dough and loaf characteristics, whereas the addition of bromate to the aqueous germ suspension caused but little improvement. Aqueous germ suspensions, on standing, lost but little of their injurious effects, but the improvement was somewhat greater when comparatively large amounts of bromate were added to them. Prefermentation of the germ caused its injurious effect on dough to disappear gradually as the time of prefermentation increased. The maximal improvement was reached after 4.5 hours' prefermentation and did not change with more extended prefermentation time. The presence of bromate in the fermenting germ suspension added to the improvement caused by prefermentation. Long prefermentation and/or long dough fermentation, particularly with bromate added at the dough stage, resulted in overmature loaves. The amino nitrogen and reducing matter content of aqueous germ suspensions increased with time of standing, and bromate tended to counteract these increases. The amino nitrogen of fermenting germ suspensions was found to be utilized by yeast. The reducing matter content of the suspensions, particularly when bromate was present, increased much less than it did in nonfermenting suspensions. While the nitroprusside reaction of prefermented germ suspensions became negative, their reducing matter content reached a level equal to or higher than that obtaining in the initial stage when the nitroprusside reaction was strongly positive. From the observation that bromate is much more effective at the dough stage than when added to a fermenting germ suspension, Smith and Geddes conclude that it exerts a direct action on flour proteins.

Laitinen and Sullivan (1941) applied the polarographic method to the study of oxidation-reduction systems in flour. They extracted

flours which had strongly responded to bromate with 0.1*N* potassium chloride or with acetate buffer solution. These extracts failed to give an anodic wave. The same negative result was obtained when the flour was treated with varying amounts of papain previous to extraction with an acetate buffer. An acetate buffer extract of wheat germ gave an anodic wave which was identified as that of reduced glutathione. Oxidation of glutathione in wheat germ resulted in a considerable decrease in the height of this wave. Unbuffered potassium chloride extracts of germ and bran yielded waves which disappeared on acidification, owing to a shift of the potential to more positive values. Extracts made from doughs (containing yeast, salt, sugar, flour, and water), with and without added potassium iodate, showed no distinctive waves. Yeast alone, extracted with acetate buffer, gave a small anodic wave. Flour extracts obtained by treating various flours with *N*/1KOH and then neutralizing with excess acetic acid so as to make an equimolecular acetate buffer, gave an anodic wave with a half-wave potential of  $-0.15$  volt (S.C.E.). The anodic waves reached their maximum height after 24 hours' extraction time with KOH. These anodic waves could be made to disappear by addition of enough potassium iodate to make the solution  $2 \times 10^{-4}M$ . This shows that the anodic wave is due to a reducing substance. Further experiments with the various flour constituents showed that this reducing substance is released by the gluten. The authors further discuss the cathodic waves caused by the presence of potassium iodate in flour extract and the polarographic performances of various acids and amino acids, and they suggest that the formation of a mercury compound may be involved in the mechanism of the anodic wave. A buffered extract of alkali-treated gluten gave a positive mercury reaction on electrolysis.

Baker, Parker, and Mize (1942) studied the action of oxidants in bread doughs by measuring the flow of doughs and by investigating the different fractions (gluten, starch, "amylodextrin," washings) obtained in the gluten washing process. The authors reached the conclusion that either the gluten or the water soluble part of the dough is responsible for the effects on dough of oxidizing agents. They found the response of gluten to physical treatment to be the same as that of dough. The property of flow could be largely removed from gluten by washing, which seems to indicate that this property is associated with water soluble constituents. The effect of papain on gluten could be reversed by extensive washing. Water solubles obtained by repeated dispersal of gluten in the Waring Blendor with a 0.75% sodium chloride solution showed many of the properties of a protease. A concentrated gluten wash solution had a softening effect on ordinary or rewashed

gluten but "tightened up" or left unchanged redispersed gluten. This was taken to show that constituents of the fraction removed by dispersal are responsible for the softening effect. It was this fraction also which was found to respond to the addition of sodium chlorite.

In view of the practical importance of the problems involved, and also for their general biochemical interest, the author thought it desirable to follow up, by quantitative methods, Hullett's and her own original experiments. The object of the present investigation was to determine (a) the effects of the germ proteases on the nitrogen distribution in the dough and (b) the effects of the —SH groups present in the germ on the —S—S— linkages of gluten.

The general plan of the study was to prepare doughs with and without addition of extracts from untreated and pretreated wheat germ, and after allowing these doughs to ferment or to stand unfermented, to wash the gluten from them and determine (a) the distribution of nitrogen between the soluble and insoluble fractions and (b) the cystine content of the washed gluten. It was anticipated that any changes effected by the action of germ proteases or germ —SH groups would be indicated by the values obtained in these determinations.

In the course of the experiments the idea emerged that dehydrogenases may play an important role in dough fermentation. In order to test this hypothesis the oxidation-reduction potential of germ extract was measured and a number of other tests were carried out.

### Materials and Methods

*Preparation of Doughs:* Doughs were made from 25 g of a commercial New Zealand flour (straight run, 72% extraction) and 15 ml of dough liquid. No salt was added, because it was feared that it might interfere with the colloidal state of the proteins present in the dough extracts. When the doughs were to be fermented, 0.5 g of yeast was suspended in the dough liquid.

*Dough Liquid:* Each experiment consisted of a series of five doughs made with different dough liquids as follows:

- (1) Extract from untreated wheat germ. This extract contained proteases and —SH groups, the determination of which will be discussed later.
- (2) Heated and centrifuged extract from unfermented germ. This extract contained —SH groups but no enzymes.
- (3) Extract from fermented germ. This extract contained enzymes but practically no —SH groups.
- (4) Heated and centrifuged extract from fermented germ. This extract contained no enzymes and practically no —SH groups.
- (5) Tap water.

The wheat germ used in these experiments was from a New Zealand flour mill and was used without any further comminution. Its content of pure germ, determined according to an unpublished method worked out by L. H. Bird at this Institute, was found to be approximately 64%.

*Preparation of Wheat Germ Extracts Used as Dough Liquids:* Extracts were prepared from this wheat germ in the following way: A mixture of 1 part of wheat germ and 2 parts of tap water, and another mixture of 1 part of wheat germ and 2 parts of yeast suspension containing 0.5 part of compressed yeast were allowed to stand for 4 hours at 30°C. They were then made up to their original weight, mixed with 4 parts of tap water, and centrifuged. Each centrifugate was divided into two portions, one of which was weighed, heated in a boiling waterbath for 3 minutes, cooled, made up to the original weight, and centrifuged. The dry solids contents of these 4 extracts were as follows:

(1) Extract from untreated germ.....	6.1%
(2) Heated and centrifuged extract from untreated germ.....	4.7%
(3) Extract from fermented germ.....	3.1%
(4) Heated and centrifuged extract from fermented germ.....	2.6%

Fifteen ml of each of these extracts was used as dough liquids without regard to the differences in their content of dry solids.

*Washing of Gluten:* After the doughs containing these extracts, or tap water, had stood for 3 hours at 27.8°C the gluten was washed from them by hand, 10 successive portions of 50 ml tap water being used for each sample. It was assumed that the total volume of combined washings would differ very little from 514 ml. This volume would be the difference between the total water, comprising (a) wash water, (b) flour moisture, (c) dough liquid, and the water remaining in the wet gluten.

When gluten was washed from unsalted doughs containing extracts from unfermented wheat germ, severe disintegration occurred and caused the bolting silk ordinarily used in the washing process to become blocked almost immediately after washing had begun. For this reason the bolting cloth was discarded altogether in all of the tests. Instead, each successive portion of washings was carefully decanted to minimize losses. The elimination of the bolting silk did, however, cause losses. While the duplicate determinations of mechanical loss agreed fairly well where water or extracts from fermented germ were the dough liquids, they showed much poorer agreement when the germ had not been fermented.

It was to be expected that proteolytic effects taking place in the dough would result in an increase of soluble nitrogen or of nonprotein

nitrogen in the washings, or in both of these factors. To detect such changes, nitrogen determinations in the washings before and after deproteinization would have been sufficient. Since, however, it was found that the use of extracts from unfermented wheat germ causes severe gluten disintegration during the washing process, it became advisable to use a method which would, in addition, estimate these losses. This was done by determining nitrogen (1) in the flour, (2) in the dough liquids, (3) in the filtered washings, and (4) in the unfiltered gluten hydrolysates. In order to include the humin nitrogen in the nitrogen determination, the gluten hydrolysates were not filtered. They were, however, well shaken before an aliquot was removed. The difference:  $[(1) + (2)] - [(3) + (4)]$  gave mechanical losses. An attempt was made to determine nonprotein nitrogen in the washings by the method of Ayre and Anderson (1939) but owing to poor duplication it was abandoned.

*Determination of —SH Groups as Reducing Matter in Germ Extracts:* Reducing matter was determined by titration with standard potassium iodate solution at a pH of 2–3. When germ extracts are acidified to such a low pH most of their protein is precipitated. After the precipitate has been filtered and washed with acid, both the filtrate and the filter residue give a strong nitroprusside reaction. The —SH groups responsible for the positive reaction of the washed filter residue seem to be of the kind which Hopkins (1925) called “fixed” —SH groups, that is, —SH groups fixed to denatured protein. In this paper they will be referred to as protein —SH groups as distinct from the “soluble —SH groups” of the acid filtrate.<sup>1</sup>

Soluble —SH groups were determined by the following technique: To 10 ml of each of the wheat germ extracts 10 ml of 10% trichloroacetic acid was added, the mixture was centrifuged, and the supernatant liquid decanted. The residue was washed and centrifuged twice with 10 ml of 3% trichloroacetic acid, and the washings added to the original supernatant liquid. This solution, after addition of 2.5 ml of 5% potassium iodide solution and 1 ml of soluble starch, was titrated with  $M/600$  potassium iodate solution.

To determine the protein —SH groups, the washed residue in the centrifuge flasks was dissolved in 10 ml of urea solution (1 : 1). This solution, acidified with a few drops of concentrated HCl, was titrated with  $KIO_3$  in the same way as the solution containing the soluble —SH groups.

At first, the germ extracts were made completely ready for the doughs on the day before dough making and were kept in the refrigerator.

<sup>1</sup> Hullett and Stern (1941) wrongly assumed that the positive nitroprusside reaction of the precipitated germ protein was due to adsorption.

tor overnight, but it soon became clear that the  $-SH$  content of the unheated extract decreased overnight, while that of the heated one remained unchanged. For this reason the unheated extracts which, for lack of time, had to be prepared the day before they were to be used, were kept in the refrigerator overnight but the heating and centrifuging were left until shortly before the particular doughs were made. The determination of  $-SH$  groups in each extract was made shortly after the corresponding dough had been mixed. Table III gives the results of these titrations.

In one series of germ extracts, reducing matter was determined by adding an excess of 0.005*N* iodine solution and back-titrating with standard thiosulfate solution. The values differed from those resulting from titration with iodate but they had the same trend.

*Cystine Determination in Washed Gluten:* Initially an attempt was made to determine  $-SH$  groups present in the filtered hydrolysates of the glutens washed from the doughs. As was to be expected, however, this approach was unsuccessful because, according to Lugg (1933), under the conditions of acid hydrolysis, the bulk of the cysteine is destroyed by formation of humin. Later, cystine was determined in these glutens because cystine should decrease with either reduction or oxidation of gluten  $-S-S-$  linkages. The technique of hydrolysis (with sulfuric acid) and colorimetry was that described by Mirsky and Anson (1935). The determination of extraneous reducing substances was carried out in the same way, except that (after Lugg, 1933) 0.5 ml of a 2.7% mercuric chloride solution was added in preparing the test solution for colorimetric reading.

*Measurement of Oxidation-reduction Potentials in Germ Extracts:* The oxidation-reduction potential of wheat germ has been measured by Potel (1935), who found  $E_h$  to be  $-169$  mv. He prepared, in an atmosphere of pure nitrogen, the suspension of 1 part of the test material with 1.5 parts of phosphate buffer of pH 6.2, added 20 ml of toluene, and protected the mixture from air by covering it with a layer of vaseline oil. The measurement was made by means of a platinum electrode at 30°C. A stable equilibrium was reached after approximately 2 hours.

The measurements reported in this paper were carried out by means of a Coleman platinum electrode as follows: Five ml of unheated germ extract was adjusted to pH 6.6 with phosphate buffer, made up to 25 ml and a few drops of toluene were added. A stream of pure nitrogen was bubbled (at a speed of roughly 5 bubbles per second) through the electrode vessel containing the extract. The temperature was 17°C. Readings became constant after about 20 hours.

*Response of Gluten to Heated and Unheated Germ Extract:* Two



samples of gluten, each washed from the equivalent of 25 g of flour, were thoroughly kneaded for 5 minutes with 15 ml of germ extracts 1 and 2 respectively. After 5 minutes the gluten samples were washed with five successive portions of tap water, formed into balls, and placed on a glass sheet resting on graph paper. In order to avoid drying out, each sample was covered with a tumbler. Two diameters, at right angles to each other, of the gluten balls were measured by means of the graph paper immediately after washing and at various intervals thereafter and the areas calculated from the mean radii.

*Detection of Dehydrogenases in Germ by the Thunberg Technique:* One-half g of germ was mixed with 2 ml water and 0.4 ml of a 0.005% methylene blue solution in a Thunberg tube which was then evacuated and placed in a thermostat at 30°C. A second Thunberg tube containing the same quantities of wheat germ and water was kept in a boiling waterbath for 15 minutes and cooled to room temperature. Then 0.4 ml 0.005% methylene blue was added and the tube was evacuated and placed in the thermostat alongside the first tube.

## Results

The results of the nitrogen determinations given in Table I show that the use of the extract from untreated germ as a dough liquid in

TABLE I  
EFFECT OF WHEAT GERM EXTRACTS ON N DISTRIBUTION IN  
VARIOUS DOUGH FRACTIONS

	Dough liquid									
	Untreated germ extract		Heated, centrifuged extract from unfermented germ		Extract from fermented germ		Heated, centrifuged extract from fermented germ		Water	
	Dough									
	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast
N in 25 g flour, mg	346	346	346	346	346	346	346	346	346	346
N in 15 ml dough liquid, mg	60	60	24	24	32	32	22	22	—	—
Total N in dough, mg	406	406	370	370	378	378	368	368	346	346
Soluble N in washings, mg	93	106	81	102	83	104	77	95	58	93
N in gluten hydrolysate, mg	173	101	199	102	266	217	257	201	255	204
Lost N, mg	140	199	90	166	29	57	34	72	33	49
Lost total N <sup>1</sup> %	34.5	49.0	24.3	44.9	7.7	15.1	9.2	19.5	9.5	14.2
Lost gluten N <sup>2</sup> %	44.7	66.3	31.1	61.9	9.8	20.8	11.7	26.4	11.4	19.3

<sup>1</sup> Loss of gluten nitrogen expressed as percent of total nitrogen.

<sup>2</sup> Loss of gluten nitrogen expressed as percent of gluten nitrogen.

unsalted nonyeasted doughs led to a severe breakdown of the gluten; this is indicated by high mechanical loss in gluten washing. The loss was only slightly lessened when the extracts were heated and centrifuged. However, in those doughs for which water or extracts from fermented germ had been used the loss of gluten was very much smaller; this held whether or not extracts had been heated and centrifuged. These results, therefore, show that fermentation of germ greatly lessens the disruptive effect of its extract on the dough. In the yeasted doughs the losses were much higher throughout than in the nonyeasted series, but the trend was the same.

Table I shows further that the soluble nitrogen in the washings from the yeasted blank is higher by 60% than that in the washings from the nonyeasted blank. Amos (1931), Freilich and Frey (1943), and Shen and Geddes (1942) have shown that nitrogen compounds are utilized by actively fermenting yeast, and for this reason concluded that the soluble nitrogen contained in a fermenting dough is unsuitable as a measure of proteolytic activity. In the present experiments, notwithstanding the partial consumption of soluble nitrogen by the yeast, an increase of soluble nitrogen occurred. This observation confirmed similar ones made by other workers.

The inclusion of wheat germ extracts in the dough, whether or not the germ had been fermented and/or the extracts heated, led to higher figures for soluble nitrogen in the washings, as compared with the blank. That no real increase, but in fact a decrease, takes place can be seen when allowance is made for the soluble nitrogen present in the dough liquids (Table II).

TABLE II  
EFFECT OF WHEAT GERM EXTRACTS ON THE FORMATION OF  
SOLUBLE NITROGEN IN DOUGHS

	Dough liquid									
	Dough liquid				Dough					
	Untreated germ extract	Heated, centrifuged extract from unfermented germ	Extract from fermented germ	Heated, centrifuged extract from fermented germ	Water					
	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast
Sum of soluble N in blank + dough liquid, mg	118	153	82	117	90	125	80	115	58	93
Soluble N found, mg	93	106	81	102	83	104	77	95	58	93

The values in Table II show that the incorporation of germ extract into dough did not result in making soluble any proteins that were insoluble in the blank. The striking fact that there was an actual decrease of soluble nitrogen when extracts of untreated germ were used, and that a similar tendency was noticeable in the case of the other germ extracts, will be discussed later. That the flour and the germ did, however, contain proteases is shown from the following evidence: Determination of protease activity as described in Cereal Laboratory Methods (4th ed. 1941) gave increases of 12 mg amino nitrogen per 100 g of flour, and 42 mg per 100 g of germ after a digestion of 24 hours at 40°C. The soluble nitrogen of a nonyeasted dough made with tap water increased by 15 mg during a standing period of 3 hours at 27.8°C.

The data in Table III show that when extracts from unfermented germ are heated and centrifuged the bulk of the protein carrying the

TABLE III

SOLUBLE AND MASKED —SH GROUPS PRESENT<sup>1</sup> IN WHEAT GERM EXTRACTS  
(Expressed in ml  $M/600$  KIO<sub>3</sub> consumed by 15 ml dough liquid)

	Nature of dough liquid <sup>1</sup>			
	Untreated germ extract	Heated, centrifuged extract from unfermented germ	Extract from fermented germ	Heated, centrifuged extract from fermented germ
Soluble —SH groups	2.01	1.86	0.12	0.17
Protein —SH groups	2.30	0.22	0.60	0.07
Total	4.31	2.08	0.72	0.24

<sup>1</sup> The headings containing the word "centrifuged" refer to the centrifuging carried out to remove protein coagulated by heating. They do not refer to the centrifuging which all extracts underwent after addition of trichloroacetic acid.

protein —SH groups is removed, but the soluble —SH groups remain practically undiminished. It can further be seen from these figures that a 4 hours' fermentation of germ at 27.8°C results in the disappearance of the bulk of the soluble —SH groups and in a considerable decrease of the protein —SH groups in the extracts. When extracts from fermented germ are heated and centrifuged, the protein —SH groups are removed. Thus, fermentation, heating, and centrifuging results in a far-reaching decrease of both soluble and protein —SH groups.

The removal by heating and centrifuging of the bulk of the protein —SH groups from an extract of unfermented germ did not appreciably decrease its injurious effect on dough, although its total content of

reducing matter had dropped to approximately half of its original value. This indicates that only the soluble —SH groups cause dough deterioration. Table IV, combined from Tables I and III, shows the

TABLE IV  
RELATION BETWEEN SOLUBLE —SH GROUPS IN DOUGH LIQUIDS AND  
LOSSES OF GLUTEN IN THE WASHING PROCESS

	Dough liquid									
	Untreated germ extract		Heated, centrifuged extract from unfermented germ		Extract from fermented germ		Heated, centrifuged extract from fermented germ		Water	
	Dough									
	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast
Soluble —SH groups	2.01	2.01	1.86	1.86	0.12	0.12	0.17	0.17	—	—
Loss of gluten-N in washing, %	44.7	66.3	31.1	61.9	9.8	20.8	11.7	26.4	11.4	19.3

correlation between soluble —SH groups in the dough liquid and gluten disintegration in terms of loss in washing.

TABLE V  
EFFECT OF WHEAT GERM EXTRACTS ON CYSTINE CONTENT OF GLUTEN

	Dough liquid									
	Untreated germ extract		Heated, centrifuged extract from unfermented germ		Extract from fermented germ		Heated, centrifuged extract from fermented germ		Water	
	Dough									
	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast	No yeast	Yeast
Cystine in recovered gluten, mg	30.8	13.0	33.6	14.4	48.5	30.2	47.3	29.2	46.1	29.7
Cystine in total gluten, mg	55.7	38.6	48.8	37.8	53.8	38.1	53.6	39.7	52.0	36.8

The data in Table V show that fermentation, but not addition of germ extract, causes a significant decrease in the cystine content of gluten.

The values in the second line of Table V were computed on the assumption that the cystine content of the coherent part of the gluten

is the same as that of the disintegrated fraction. If this is true, the cystine determined in the hydrolysate of the coherent fraction should bear the same relation to the cystine, present but not determined, in the disintegrated part as the nitrogen contents of these two gluten fractions bear to each other. The total cystine content of gluten would thus be:  $\frac{\text{cystine found}}{100 - \% \text{ lost gluten}}$ . Where the figures calculated on this basis

differ significantly from those referring to the nonyeasted blank, either a change in the cystine content of the gluten or of cystine distribution (between coherent and disintegrated fractions) must have taken place as a consequence of the particular treatment.

The results of the experiments, which related to the effects of dehydrogenases, were as follows:

The oxidation-reduction potential of untreated wheat germ extract was found to be  $-115$  to  $-130$  mv; that of fermented wheat germ extract  $-150$  to  $-160$  mv.

Wheat germ, tested by the Thunberg technique, was found to contain dehydrogenases; the unheated germ suspension decolorized methylene blue in 5 minutes at  $30^{\circ}\text{C}$  whereas a similar suspension which had been previously heated in a boiling waterbath for 15 minutes did not decolorize methylene blue.

When gluten was treated with the germ extracts 1 and 2, the rates of flow as reflected by the relative areas of the gluten surfaces in contact with graph paper were as follows:

Areas	Immediately after washing	After 1 hr	After 2 hr	After 3 hr	After 5 hr	After 6 hr
Extract 1 (untreated)	64	82	95	113	118	133
Extract 2 (heated, centrifuged)	78	118	148	165	177	177

### Discussion

The doughs used in the experimental part of this investigation are not comparable to ordinary bread doughs, mainly because they do not contain salt. Therefore the following views, which were suggested by the experimental results, will have to be checked by evidence obtained with ordinary bread doughs.

The determination of the nitrogen distribution in doughs revealed the interesting fact that neither the addition of proteases nor of  $-\text{SH}$  groups resulted in an increase of soluble nitrogen but that, on the

contrary, there was a decrease of soluble nitrogen. This latter fact can only be explained by the assumption that the gluten incorporated some of the protein from the dough liquids. Something of the same nature seems to take place in the well-known case where an increased yield of gluten is obtained by washing gluten from a mixture of wheat and rye flour.

Shen and Geddes (1942) give a survey of the evidence used by various workers in support of Jørgensen's hypothesis (activation of flour proteases by —SH groups) and of the hypothesis that —SH groups exert a direct action on gluten. It may be of interest to investigate how the results reported in this paper and some of the earlier evidence fit in with either of these hypotheses.

If Jørgensen's view is correct, the injurious effect of unheated germ extract could be explained as a consequence of adding —SH groups and germ proteases to the dough. The injurious effect of heated germ extract may also be attributed to —SH groups acting on the flour proteases, but one would expect that the inactivation of the germ protease in the dough liquid would result in lessening the damage to some extent. Actually, the damage (expressed in terms of loss in washing and increase of soluble nitrogen) caused by the heated germ extract was less severe than that due to unheated germ extract. Also, the good characteristics of dough made with germ extracts (heated or unheated) from fermented germ may be explained by the absence of —SH groups. This experiment, however, cannot be regarded as convincing evidence in favor of Jørgensen's hypothesis, for the reason that it supports the direct-action hypothesis equally well.

This latter hypothesis does not deny that proteases are present in flour and that they belong to the papain type which is activated by —SH groups but it claims that —SH groups also have a direct effect on gluten; *e.g.* when gluten is treated with cysteine. On the basis of this hypothesis, the effects on doughs of the wheat germ extracts described in the experimental part may be explained as follows: Unheated extract from untreated germ has a proteolytic plus a direct effect; after heating it has a direct effect only. In extracts from fermented germ the protease is inactivated as no —SH groups are present; similarly after heating, the protease is destroyed and no —SH groups are present. Thus, this experiment, while giving information on changes in gluten structure, is not helpful in judging the validity of either of the two hypotheses.

One of the main objections to Jørgensen's hypothesis is the fact that prolonged fermentation of doughs injured by —SH compounds leads to a very considerable improvement of dough and loaf characteristics. Such an improvement with time is not compatible with the

idea that the initial damage is caused by proteases. As Hullelt (1940) was the first to suggest, the elimination of —SH groups from a fermenting dough may be an important part of the ordinary dough-ripening process. The work of Smith and Geddes (1942) also makes it appear probable that the improving effect of long fermentation is due to the destruction of —SH groups. While this gradual disappearance of —SH groups may cause a gradual inactivation of proteases, it is quite unlikely to reverse chemical changes brought about by proteolysis in the earlier stages of fermentation.

If, on the other hand, one tries to interpret the observations on the basis of the direct-action hypothesis, one arrives at the conclusion that this direct action must be of such a nature that, by the oxidation of the —SH groups, it allows gluten structure to be restored to a normal condition. Otherwise the improvement of dough characteristics with length of fermentation could hardly be explained.

The abnormal response of gluten to treatment with unheated and heated germ extracts shows that a heat-labile factor has a tightening effect on gluten, whether damaged by —SH groups or not. Obviously, heating the germ extract destroys some factor which favorably affects gluten structure or lessens the damage due to —SH groups. Sullivan, Near, and Foley (1936) found that wheat germ stored in closed containers at relatively high moisture content loses its injurious effect. Hullelt and Stern (1941) and Smith and Geddes (1942) showed that fermentation causes a much more rapid elimination of these damaging properties, and the first-named authors found that an enzyme contained in the germ was essential in this process. Both storage and fermentation of wheat germ are accompanied by the disappearance of the nitroprusside reaction, and in both cases it was found impossible to restore the nitroprusside reaction by means of reducing agents.

On the basis of these findings the author assumed that the enzyme responsible for the elimination from germ of the —SH groups is a dehydrogenase and that the oxidation of the —SH groups proceeds beyond the —S—S— stage. The observation that cyanide does not inhibit the destruction of —SH groups by fermentation (Hullelt and Stern, 1941) fits in with the assumption that a dehydrogenase is effective in this process.

Dehydrogenases are present in many seeds, as Thunberg has found. The present study indicates that they are present in wheat germ in considerable amount. Since every flour contains some germ, the amount varying with the length of extraction, it seems reasonable to expect that mechanisms analogous to those playing a part during storage and fermentation may be active in flour and dough. Fer-

mentation speeds up the otherwise slow action of the dehydrogenases. Nothing is known so far about the mechanism of this acceleration except that the glutathione present in the yeast cell enters into it, as was found by Hullett and Stern (1941).

By oxidizing  $\text{—SH}$  compounds, the dehydrogenases would bring about the aging of green flour and the maturing of dough. The gradual elimination of  $\text{—SH}$  groups by dehydrogenases would explain the improving effect of prolonged fermentation of low-grade flours. In the gluten experiment the presence of dehydrogenase in the unheated extract would tend partly to offset the damage caused by  $\text{—SH}$  groups and proteases.<sup>2</sup>

Sullivan *et al* (1940) suggested that dough fermentation, reducing and oxidizing agents, and physical manipulation may bring about changes in the sulfur linkages of the gluten proteins. The results reported above showed that, under the conditions applied, fermentation produced a significant decrease of cystine in the gluten, whereas the presence of wheat germ extracts rich in  $\text{—SH}$  groups failed to produce a significant change in the cystine content of the gluten.

As has been discussed above, it is difficult to explain the improving effect of prolonged fermentation on dough made from low-grade flours unless one assumes the direct action of  $\text{—SH}$  groups to be of such a nature that it allows gluten structure to be restored to normal condition. The cystine determinations reported in this paper indicate that the addition of  $\text{—SH}$  compounds had no significant effect on the  $\text{—S—S—}$  linkages of the gluten. This shows that the  $\text{—S—S—}$  linkages were not involved in whatever reaction may have taken place owing to the direct action of  $\text{—SH}$  groups. The fact that dough fermentation leads to a considerable decrease of cystine in gluten confirms the views of those investigators who maintain that yeast is not only a leavening agent but produces fundamental biochemical changes in the dough.

### Summary

Wheat germ extracts contain soluble  $\text{—SH}$  groups and  $\text{—SH}$  groups attached to protein.

The distribution of soluble and protein  $\text{—SH}$  groups in heated and unheated extracts from unfermented and fermented wheat germ is reported.

The soluble  $\text{—SH}$  groups were found to cause the injurious effect of wheat germ extract on dough.

Fermentation of wheat germ decreases its content of soluble and protein  $\text{—SH}$  groups.

<sup>2</sup> This experiment also suggests that the dehydrogenase must have exerted a considerable effect within the 5-minute contact of the gluten with the germ extracts.



Extracts from unfermented wheat germ, when added to unsalted dough, cause severe gluten disintegration on washing.

Dough fermentation increases the gluten disintegration in the blank doughs as well as in those containing germ extracts.

There is no increase, but an actual decrease, of soluble nitrogen in doughs containing extracts from unfermented germ.

Dough fermentation leads to a decrease of cystine in the gluten, but —SH groups did not significantly affect the cystine content of the gluten.

Aging of flour and maturing of dough is assumed to be connected with the oxidation of the —SH groups of germ particles contained in the flour.

The oxidation of —SH groups in stored flour and fermenting dough is attributed to dehydrogenases, and the presence of dehydrogenases in wheat germ is demonstrated.

Reasons are given for the assumption that the injurious effect of —SH groups is direct but of such a nature that it will allow the gluten to be restored to normal, that is, to the condition obtaining in fermented loaves made from high grade flours.

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## THE ACTION OF OXIDIZING AGENTS ON SULFHYDRYL COMPOUNDS IN DOUGH

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This paper reports further studies on the effects of oxidizing agents on refined flour and dough. The work has been guided by our previous researches (Baker, Parker, and Mize, 1942; Baker, Mize, and Parker, 1943), in which it was found that oxidizing agents cause changes in puncture strength of gluten and in properties of soluble pentosan and soluble protein. While, admittedly, effects of oxidizing agents upon starch, other carbohydrates, and lipoids should be studied further, it seemed that continued studies of the proteins were more promising, especially since it had been found that glutathione lowered the resistance of gluten to puncture and influenced the viscosity of water soluble pentosans. Also, as many workers have shown (Balls and Hale, 1936; Jørgensen, 1936; Sullivan, Howe, and Schmalz, 1936, 1937; Ford and Maiden, 1938; Freilich and Frey, 1939; Ziegler, 1940, 1940a; Hullett and Stern, 1941; and others), glutathione profoundly affects dough and its bread making properties.

Sullivan, Howe, and Schmalz (1936) isolated glutathione from wheat germ but reported that patent flours contained no glutathione as indicated by the nitroprusside test. Hence, it could be assumed that there were no other available sulfhydryl compounds present in patent flour. Balls and Hale (1936) have shown that a positive nitroprusside

test can be obtained from petroleum ether extracts of patent flour and have suggested that sulfhydryl groups may be present in flour protein. They have also isolated a water soluble sulfur-containing polypeptide from the petroleum ether extract. These observations, together with the fact that sulfhydryl compounds are easily oxidized, suggest that the baking properties of flours may be influenced by their sulfhydryl content, particularly in regard to their behavior toward oxidizing agents.

Greenstein (1938) and Anson (1941) have published methods for the determination of -SH groups in proteins. Greenstein (1938) showed that sulfhydryl groups, which cannot be detected in certain native proteins (such as egg albumin, edestin, excelsin, and globins), are present in these proteins after denaturation with guanidine hydrochloride and urea, or their derivatives. He titrated the -SH groups with porphyrindin as the oxidizing agent employing sodium nitroprusside as the end point indicator. Anson used either sodium tetrathionate or potassium ferricyanide in place of porphyrindin. The end point is the least amount of ferricyanide or tetrathionate which will prevent the formation of the pink color, thus indicating the amount required to oxidize the sulfhydryl groups.

In the present study, the sulfhydryl contents of aqueous extracts and glutens, prepared from highly refined flours of varying strength, were determined. The effect of oxidizing agents on the -SH content of these flour and dough fractions was investigated. The puncture strength of the glutens was also determined to ascertain whether any relation exists between gluten strength and -SH content.

### Experimental

*Determination of sulfhydryl groups.* Preliminary studies showed that the use of guanidine hydrochloride as a solvent in determining the sulfhydryl content of flours and doughs was unsatisfactory. The starch present gave a highly viscous solution which made mixing of the reagents difficult and also interfered with the color reaction so that the end point could not be determined. A modified Anson (1941) method, however, was found to be satisfactory when applied to concentrated extracts of flour or dough (flour-water ratio, 1 : 2) prepared as described by Baker, Mize, and Parker (1943). It also gave very good results with aqueous dispersions of gluten. To disperse the glutens for sulfhydryl determinations it was necessary to use less salt than formerly described; the doughs were mixed with 0.75% NaCl solution and the glutens washed therefrom with 0.15% NaCl solution. The glutens thus prepared from refined flours could be dispersed in distilled water with the Waring Blender to a stable milklike emulsion of 2.0% protein content, which made accurate sampling possible.

The method of determining -SH groups, as used in this study, is outlined below.

- Reagents:*
1. Buffer—equal parts 1.0*M* disodium phosphate, and 1.0*M* monosodium phosphate.
  2. Potassium cyanide solution, 0.1*N*.
  3. Guanidine hydrochloride—highest purity.
  4. Sodium nitroprusside solution, 5%. This is best prepared from ground sodium nitroprusside. It should be freshly made each day and stored at 0° C away from direct sunlight.
  5. Concentrated ammonium hydroxide, 27%.
  6. Potassium ferricyanide; standard solution series (0.000025*M* to 0.0008*M*, in increments of 0.0001*M*).

*Procedure:* The titration of the -SH groups is carried out by means of a series of test tubes in each of which is placed 0.5 ml of flour extract or gluten suspension, 0.1 ml of buffer solution, 0.05 ml of 0.1*N* potassium cyanide, and 0.5 ml of standard potassium ferricyanide solutions of varying strengths. Next, 1.2 g of guanidine hydrochloride (Eastman) is added; the tubes are shaken lightly to dissolve the guanidine hydrochloride, and then immersed in a water bath at 37° C for 3 minutes, followed by cooling in an ice bath. When cool, 0.05 ml of nitroprusside reagent is added, shaken, and followed by 0.05 ml of 27%  $\text{NH}_4\text{OH}$ . By selection of the proper pipette tip 0.05 ml can be delivered with one drop. The contents of the tubes are now mixed and the end point is taken as the concentration of ferricyanide where the pink color just fails to develop. From the quantity of ferricyanide required, as checked against glutathione standards, the -SH, or glutathione, content of the flour extract can be calculated.

Since the -SH values are in the lower range of Anson's scale, great accuracy cannot be expected. Although the method is fairly accurate and reproducible when applied to soluble extracts or dispersed gluteins from refined flours, lower grade flour extracts or gluten suspensions appear to have materials present which interfere with the end point, causing rapid fading and off-shades of color. A further limitation of the method was found in studying fermented doughs and gluteins prepared therefrom; yeast or its by-products interferes somewhat with the duplication of results. Anson prescribes very dilute cyanide to remove interfering effects of heavy metals. We have used this precaution in our determinations. The small amount of cyanide used does not increase the readings, indicating that there is no splitting of S-S groups. Anson recommends the use of reagents of highest purity for sharpest end points.

*Action of oxidizing agents in flour.* The sulfhydryl contents of water extracts, hand-washed gluteins, and purified gluteins from a series of commercial flours of widely varying strength are given in Table I, together with the puncture strength of the gluteins. The nitrogen contents of the fractions were determined and the -SH values are expressed on a unit protein basis.<sup>1</sup>

The -SH content per unit of protein in the water extract of the different flours is variable. The glutathione content of the solubles

<sup>1</sup> This method of expressing our results is not intended to imply that the -SH so recorded is thus part of the protein compound.

TABLE I  
SULFHYDRYL CONTENT OF WATER EXTRACTS AND GLUTENS FROM  
VARIOUS WHEAT FLOURS

Description of flour	Ash	Protein	Soluble protein	-SH per gram protein			Grams to puncture gluten	
				Water extract	Hand-washed gluten	Purified gluten	Gluten washed in 0.15% NaCl	Gluten purified in 0.15% NaCl
	%	%	%	mg	mg	mg	g	g
Montana	0.51	14.2	1.9	1.22	0.22	0.13	10.0	19.7
Nebraska	0.40	11.9	1.7	0.88	0.20	0.09	8.5	16.7
Durum	0.61	12.0	1.7	1.14	0.19	0.12	4.8	10.1
No. Spring	0.39	12.9	1.6	0.83	0.17	0.10	10.5	24.9
Soft Winter	0.31	8.2	1.3	0.73	0.12	0.10	10.7	19.5
Kansas	0.39	12.2	1.7	0.89	0.17	0.10	6.6	14.2
Pacific Pat.	0.41	10.5	1.5	0.87	0.15	0.12	6.1	14.6
Pacific Clear	0.73	12.2	2.5	1.31	—	—	4.9	11.6
Idaho	0.41	11.2	2.0	1.01	0.12	0.05	7.2	14.5

doubtless depends upon the amount of germ milled into the flour. There may be other -SH bearing compounds present, such as polypeptides, which are intermediate between glutathione and protein. The relative amounts of such compounds could account for the varying sulfhydryl content per unit protein of these soluble extracts.

The hand-washed glutens show a considerable variation in sulfhydryl content. In purified gluten, since dispersion washing by the method previously described by us has further removed soluble sulfhydryl compounds there appears to be -SH which cannot be removed by washing. There is no relation between the glutens and their sulfhydryl contents or the sulfhydryl content of the water extract.

Table II shows the effect of relatively large treatments of the Montana flour listed in Table I with nitrogen trichloride and chlorine on the sulfhydryl content and puncture strength of the glutens. These oxidizing agents lowered the sulfhydryl of the soluble extract about 20%, and of the purified gluten over 30%. If one assumes that glutathione is the compound in the soluble extract which has been oxidized, the decrease in -SH is equivalent to 36 ppm of glutathione. More than this amount is actually oxidized in the flour because none of the soluble -SH which is retained by the starch or gluten is accounted for by this calculation. The difference in -SH content of the hand-washed glutens from untreated and heavily nitrogen-trichloride-treated flour is equivalent to 56 ppm of glutathione. This value, together with the calculated equivalent glutathione in the water extract, totals about 92 ppm glutathione and is sufficient to account for most of the changes observed in flour treated by flour oxidants.

TABLE II  
EFFECT OF FLOUR OXIDATION ON SULFHYDRYL CONTENT OF  
WATER EXTRACT AND GLUTEN

Treatment	-SH per gram protein			Grams to puncture gluten	
	Water extract	Hand-washed gluten	Purified gluten	Gluten washed in 0.15% NaCl	Gluten purified in 0.15% NaCl
	mg	mg	mg	g	g
MONTANA FLOUR					
Untreated	1.22	0.22	0.13	10.0	19.7
Nitrogen trichloride, 6 g/bbl	0.95	0.16	0.09	9.8	20.0
Chlorine, 2 oz/bbl	0.98	0.17	0.09	9.0	21.9
IDAHO FLOUR—NATURAL AGE					
Stored 6 mo refrigerator	1.01	—	0.05	7.2	14.5
Stored 6 mo room temp	0.84	0.12	0.04	6.5	14.9

Experimentally, we have found that 50 ppm of glutathione will cause a dough from an optimum nitrogen-trichloride-treated flour to revert to the "green" characteristics of its untreated control, while 100 ppm of glutathione will usually produce a "green" dough when added to an over-treated flour.

The hand-washed gluten from an untreated flour, upon purification by dispersing four times in a Waring Blendor, has its reactive -SH lowered from 0.22 to 0.13 mg/g of gluten; in treated flours it has been lowered to 0.09 mg. The residual -SH is not free glutathione since glutathione added to gluten or to flour can be removed by thorough dispersion washing. The reaction of flour oxidants upon the sulfhydryl of purified glutens might account for the alteration of the gluten properties. However, the puncture values of such glutens, as shown in the last column, are not altered appreciably by the treatment.

In Table II the naturally aged flours show changes in -SH similar to those produced by nitrogen trichloride and chlorine.

There are doubtless other effects of oxidizing gases on the flour than those indicated by these -SH analyses. The petroleum-ether-soluble -SH-containing compound reported by Balls and Hale (1940) was found by them in sufficient amount to account for the above changes in -SH in flour bleaching.

Extraction of flour with volatile fat solvents lowered the sulfhydryl content of the flour fractions to approximately the same extent as is

shown by the gas-treated flours in Table II. However, treatment of such extracted flours with dough oxidants shows a further lowering of the -SH content of both water extract and gluten, indicating that there are reactive sulfur compounds in flour other than those which are removed by fat solvents.

*Action of oxygen on doughs.* A study of the action of molecular oxygen is shown in Table III. The Montana flour referred to in

TABLE III  
EFFECT OF MIXING DOUGHS IN OXYGEN AND CARBON DIOXIDE ON SULFHYDRYL CONTENT OF WATER EXTRACT AND GLUTEN  
(Montana Wheat Flour)

Duration of mix	Gas	-SH per gram protein			Grams to puncture gluten	
		Water extract	Hand-washed gluten	Purified gluten	Gluten washed in 0.15% NaCl	Gluten purified in 0.15% NaCl
min		mg	mg	mg	g	g
8	CO <sub>2</sub>	1.22	0.22	0.13	10.0	19.7
8	O <sub>2</sub>	1.07	0.21	0.09	11.9	23.1
16	O <sub>2</sub>	0.91	0.14	0.09	12.4	22.5
24	O <sub>2</sub>	0.84	0.13	0.09	12.4	23.3
40	O <sub>2</sub>	0.84	0.15	0.09	10.0	17.5
40	CO <sub>2</sub>	1.12	0.22	0.13	10.8	20.5

Table II was mixed in the presence of pure oxygen for periods of time varying from 8 to 40 minutes in a covered McDuffy mixing bowl. Similar mixings in carbon dioxide for an 8- and a 40-minute period indicate that the mixing itself had substantially no effect upon the sulfhydryl of the water extract or of the gluteins. Oxygens, however, progressively lowered the -SH content of the water extract as the mixing was continued up to 24 minutes. Further mixing produced no change, indicating that the -SH in the water-soluble portion of the dough is susceptible to attack to a limited degree. The first 8 minutes of mixing in oxygen decreased the -SH content of the gluten, after which no further decrease occurred.

The puncture values reached a maximum after 8 minutes of mixing in oxygen, and remained substantially constant up to and through 24 minutes of mixing, *but* mixing for 40 minutes materially lowered the strength of the gluten. The sulfhydryl figures, however, do not suggest this change. Doubtless, the lowering of the protein strength is due to some action other than on the -SH groups. These data again indicate that there is an oxidizable form of sulfur in gluten which is not readily removed by washing.

*Action of sodium chloride in doughs.* A further study of the effect of dough oxidants is shown in Table IV. Dough was mixed for 8 minutes

TABLE IV

EFFECT OF DOUGH OXIDATION WITH SODIUM CHLORITE ON SULFHYDRYL CONTENT OF WATER EXTRACT AND GLUTEN  
(Doughs Prepared from Kansas Flour)

Sodium chlorite	-SH per gram protein			Grams to puncture gluten	
	Water extract	Hand-washed gluten	Purified gluten	Gluten washed in 0.15% NaCl	Gluten purified in 0.15% NaCl
<i>ppm</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>g</i>	<i>g</i>
0	0.89	0.17	0.10	6.6	14.2
2.5	0.84	0.13	0.09	5.7	17.5
5.0	0.79	0.14	0.09	7.4	18.2
10.0	0.79	0.16	0.09	8.2	15.7
20.0	0.79	0.14	0.09	6.1	14.1
40.0	0.73	0.17	0.09	2.8	9.9
80.0	0.61	0.18	0.09	1.8	7.0

in the presence of sodium chlorite in amounts up to 80 ppm. Our supply of the Montana flour being exhausted, these experiments were performed on a Kansas flour. This flour contained less water soluble -SH than the Montana flour and also less -SH in the hand-washed and purified gluten. Sodium chlorite, the oxidant used in this experiment, decreased sulfhydryl content of the water extract less than did the oxygen or the gas treatments with the Montana flour used in the previous two experiments. This Kansas flour apparently has a lower proportion of oxidizable sulfur in the water extract than the Montana flour. A powerful oxidizing agent, such as sodium chlorite, would be expected to produce a greater effect than molecular oxygen or flour oxidants.

As in the previous experiment, an -SH level in the water extract is reached which is decreased by further oxidation with difficulty. Over the range of treatments from 5 ppm to 20 ppm no change occurred and the puncture strength of the glutens maintained a fairly high figure. Heavier treatments with sodium chlorite slightly lowered the -SH content of the water extract, and at the same time lowered the puncture strength of the gluten. It is difficult to associate puncture strength of gluten with the constituents of the water extract unless the strength of the former is determined by adsorbed materials. However, the thoroughly washed, purified glutens from this flour suggest no such change in the adsorbed materials. Neither is there much effect of the chlorite upon the sulfhydryl of the glutens. With this flour, nearly all the sulfhydryl sulfur could be washed from the gluten, resulting in constant values for the purified gluten throughout the whole range of the treatments. This value is only slightly lower than the -SH in the untreated purified gluten. The purified glutens of these two flours



(Montana and Kansas) indicate a difference in the amount of -SH adsorbed in the gluten and in the ease with which the -SH may be removed by washing. After oxidation and thorough washing, the amount of residual -SH in the glutens from the two flours reaches almost the same value over the total range of the respective treatments, indicating that the amount of nonoxidizable -SH in the purified glutens in these widely different flours is approximately the same. Other flours may have glutens which retain less -SH as shown by the naturally aged Idaho flour in Table II.

The baking properties of these doughs appear to be associated more definitely with the strength of the gluten than with their sulfhydryl content. Gluten puncture strength was increased by the use of sodium chlorite up to 5 ppm and then steadily decreased with increasing dosages. These changes in protein strength closely parallel the changes in the baking properties of straight doughs given this range of treatment. Other methods of baking, such as sponge doughs and no-dough-time doughs, need the higher levels of sodium chlorite where some weakening of the gluten has occurred. Further research is needed to determine the chemical reactions responsible for the changes in the physical properties of the gluten which result from treatment with oxidizing agents.

As previously mentioned, difficulty was encountered in obtaining satisfactory results on fermented doughs. Disintegrated yeast which does not separate from the supernatants upon centrifuging, and yeast which remains in the gluten throughout the washing process, apparently causes this difficulty. In spite of these difficulties, it has been found that during fermentation bromate alters the sulfhydryl content of the water extract and the puncture strength of the gluten in a manner similar to the medium chlorite treatments shown in Table IV. Fermentation alone, as used in bread making, lowers the sulfhydryl content of the water extract to a small degree. Shen and Geddes (1942) found that the total reducing matter content of extracts prepared from nonfermenting doughs increased more with time than that of fermenting doughs; bromate had a marked depressing effect which was more pronounced the longer the fermentation.

Hullett and Stern (1941) report that extended fermentation will completely remove glutathione from germ. The slight decrease in -SH content of water extracts prepared from fermented doughs made from refined flours indicates that there is little glutathione in these flours. Hence, the change in -SH by oxidation must be due to the action of the oxidizing agents upon -SH bearing material other than glutathione. This is in agreement with the work of Sullivan, Howe, and Schmalz (1936), in which they were unable to separate glutathione from flour.

### Discussion

The nitroprusside test for indicating sulfhydryl groups is not sufficiently sensitive to indicate clearly their presence in the native flour fractions, as separated by our procedures, *viz.*, the water solubles, the crude gluten, and the purified gluten. Active sulfhydryl groups must be present in these fractions since flour and dough oxidants decrease the amounts of sulfhydryl found when determined after denaturing with guanidine hydrochloride. We have found these commercially used oxidizing compounds capable of lowering the amount of -SH found when determined in the guanidine hydrochloride solution of the three fractions of all flours. These reactions indicate that the -SH is reactive in the native flour when contacted by certain flour bleaching agents or dough improvers or by molecular oxygen acting in either flour or dough. This activity of -SH groups has also been shown by Anson (1941). He found upon treating solutions of native proteins with iodine in potassium iodide that complete oxidation of the active -SH groups was obtained, as determined after denaturing.

The purified glutens from all but two of the flours studied contain approximately the same amount of -SH. The method of determining -SH is not sufficiently accurate at these low levels nor have enough flours been studied to draw any conclusions from this apparent uniformity of composition. Nevertheless, the two exceptions shown above appear sufficient to void any conclusion, for if purified glutens were constant in their sulfhydryl composition there would be no exceptions.

It appears most likely that the active -SH groups in purified gluten are in adsorbed compounds and so strongly held that the extensive washing processes which we have used do not entirely remove them. Differences in the amount of such adsorbed compounds would lead to the variation found in the composition of the purified gluten.

### Summary

Sulfhydryl content of concentrated aqueous extracts and gluten dispersions prepared from highly refined flours may be determined by a modified Anson method. With refined flours of widely varying strength, the -SH values per gram protein ranged from 0.73 to 1.22 mg for aqueous extracts; from 0.12 to 0.22 mg for hand-washed glutens; and from 0.05 to 0.13 mg for purified glutens. No consistent relationship was found between the -SH content of the three flour fractions of various flours or between their -SH content and gluten strength. A large fraction of the -SH in the glutens could not be removed by repeated washings. Since added glutathione may be readily washed out, a considerable proportion of the sulfhydryl groups of flour is

apparently present in more complex cysteine combinations. These sulfhydryl compounds do not appear to be an integral part of the molecular structure of the gluten proteins, since the -SH content of the purified glutens varied widely. The sulfhydryl bearing compounds probably are of many compositions and include glutathione, the fat soluble sulfur bearing compound of Balls and Hale, and doubtless other compounds of more complex structure which are more strongly adsorbed by the flour proteins.

Treatment of flour with chlorine and nitrogen trichloride, mixing doughs in oxygen or with sodium chlorite, fermenting doughs with or without bromate, and natural aging of flour lowered the -SH content of water extracts and glutens prepared therefrom. The total decrease in -SH which resulted from the flour treatments was equivalent to approximately 100 ppm glutathione, a quantity which is sufficient to produce marked effects in breadmaking. Changes in gluten strength did not parallel the reductions in -SH content due to oxidation.

The reasons for the effects of -SH compounds upon the baking properties of flour are not shown by these studies. Such effects may be due to some properties similar to the peptizing action of cysteine but of a much milder nature.

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## THE EFFECT OF LEVEL OF SOIL FERTILITY ON WHEAT QUALITY<sup>1</sup>

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Plant food is being mined from all soils where crops are grown. The fertility of the soil, especially in the humid region, is rapidly declining. Wheat plants and wheat seedlings grown in this region are showing nitrogen and phosphorus deficiency symptoms, and some wheat fields even indicate potash starvation. It is essential, therefore, to investigate the influence of the level of soil fertility on the quality of the wheat crop.

Several reports have been published on the influence of various fertilizers and soil treatments on wheat composition, but few data are available on the effect of different levels of soil fertility on quality in wheat.

Working with soft winter wheats, Bayfield (1936) reported that the protein content in wheat tends to increase with increasing heaviness of texture and also with increasing fertility of the soil upon which the wheat is grown. Fisher and Jones (1931) showed that, in general, the fertilized plots produced wheat of better baking quality than the unfertilized plots. However, the order of the quality of the various plots was different every year. Results reported by Sullivan *et al* (1938) indicated that the plots which had not received any fertilizer produced wheat with shrunken grains and high protein content. When complete fertilizer was used in addition to the lime, the wheat had a lower protein content, higher kernel weight, higher test weight, and lower vitreousness than when lime alone was used.

The present study reports the quality of five varieties of wheat

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when grown on soils in which different levels of soil fertility were established by the addition of mineral fertilizers. The tests were conducted on Plainfield fine sand, Crosby silt loam, and Alford silt loam soils during the 5-year period 1937 to 1941.

### Materials and Methods

Five varieties of wheat that varied in inherent quality were selected and grown at all locations. These were, in order of ascending gluten strength, American Banner, Purdue No. 1, Trumbull, Michigan Amber, and Michikof. The three soil types—Plainfield fine sand, Crosby silt loam, and Alford silt loam—are on Purdue University experimental farms located near Culver, Lafayette, and Wheatland, Indiana, respectively. On each soil type, three levels of fertility were established by the addition of mineral fertilizers as follows: Low level, none; medium level, 250 lb of 2-12-6 (percentage of N,  $P_2O_5$ ,  $K_2O$ ) in the fall and 100 lb of sodium nitrate in the spring; high level, 500 lb of 2-12-6 and 200 lb of sodium nitrate. On the Plainfield fine sand a 3-12-12 analysis fertilizer replaced the 2-12-6.

Each level of fertility was replicated three times and each variety was replicated 15 times on each fertility level, making a total of 225 samples at each location each year. Quality analyses were made on 3150 samples obtained in this experiment. A recent publication by Worzella (1943), reporting the yield results, gives further detail as to randomization, plot size, field plan, etc., of this experiment.

Gluten strength was determined by the wheat-meal fermentation-time test as described by Cutler and Worzella (1936). Granulation (the particle-size index of wheat meal) was determined according to the method developed by Worzella and Cutler (1939, 1943). The method reported by Binnington and Geddes (1939), with some minor modifications, was used for determining the carotinoid content of wheat. A 10-g sample of finely ground wheat meal was placed in a 4-oz bottle containing 50 ml of water-saturated butanol and allowed to stand for 16 hours with occasional shaking. Clarification was effected by filtration through No. 1 Whatman paper. The yellow pigment content of the extract was determined in a 2-cm cell, using a KWSZ photometer. Pure beta-carotene was used to prepare a series of known standards. By means of a conversion table, the percentage transmittancy readings were expressed as parts of carotene per million parts of wheat meal. Crude protein and ash determinations were made according to the methods outlined in *Cereal Laboratory Methods* (4th ed. 1941), and are reported on a 13.5% moisture basis. The test weights of the small samples of wheat were determined by the method developed by Aamodt and Torrie (1934).

Except for carotinoid pigment content, protein, and ash, analyses were made on each of the 3150 wheat samples obtained in this experiment; for these particular components of quality, 630 composite samples were employed. Analysis of variance was used to aid in the interpretation of the data.

### Experimental Results

The 5 years during which these data were obtained were quite favorable for wheat growing. Winter injury did not occur to any appreciable degree in any year under consideration. Lodging was not a factor in influencing the results even on the high levels of fertility. No data were obtained on the Crosby silt loam soil in 1937 because of a severe stem rust epidemic in the Lafayette area. Except during the dry weather in April 1938 and May 1939 on the Plainfield fine sand (Culver), moisture was adequate throughout the wheat growing season each year at all locations. All varieties of wheat grown on the higher levels of soil productivity ripened from 1 to 2 days earlier than those on the low fertility plots.

The experimental data on yield, gluten strength, granulation, carotinoid pigment, crude protein, test weight, kernel size, and ash are presented in the order named. Since the experimental farms are located some 200 miles apart, factors such as climate, soil type, adaptation, etc., interfere in making a direct comparison between locations. Consequently, the data for the three locations have been summarized and analyzed separately.

*Yield.* Yield may be regarded as the ultimate expression of all environmental conditions and inherent factors that have integrated throughout the life of the plant. Grain yield results, therefore, aid in the interpretation of quality studies. The yield data, involving the samples used in this study, have been reported in a recent publication by Worzella (1943). It was shown in the above report that, on the basis of 4- or 5-year averages, the yields in bushels per acre for low, medium, and high levels of soil fertility were as follows: 14.2, 22.2, and 25.0 for the Sand Field; 19.7, 30.4, and 35.2 for the Knox County Experimental Farm; and 27.8, 35.0, and 40.7 for the Soils and Crops Farm, respectively. The yield data indicate, therefore, that the wheat samples used in these quality studies were grown on widely different soil productivity levels.

*Gluten Strength.* The data for fermentation time and analyses of variance of the five varieties of wheat grown on the three levels of soil fertility at the three locations during the 5-year period 1937-41 are reported in Table I.

TABLE I

FERMENTATION TIME FOR GLUTEN STRENGTH AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937-41

Levels of soil fertility	Fermentation time in minutes					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	38.1	41.6	25.1	48.7	185.1	67.7
Medium	43.7	44.1	27.1	54.6	200.3	74.0
High	48.8	45.3	29.6	55.1	193.7	74.5
Mean	43.5	43.7	27.3	53.0	193.1	72.1
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	33.5	36.3	22.4	44.8	158.5	59.1
Medium	35.7	38.0	23.6	50.4	176.0	64.8
High	37.8	40.6	25.8	56.2	185.2	69.1
Mean	35.7	38.2	23.9	50.5	173.2	64.3
SOILS AND CROPS FARM (LAFAYETTE) <sup>1</sup>						
Low	33.4	34.1	22.3	43.2	150.5	56.7
Medium	35.0	35.0	23.0	45.0	154.4	58.5
High	36.2	37.3	24.8	47.8	170.0	63.2
Mean	34.9	35.5	23.4	45.3	158.2	59.4
ANALYSIS OF VARIANCE						
Item	Degrees of freedom	Mean square				
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm <sup>1</sup>		
Season	4	1,183†	1,062†	645†		
Varieties	4	69,902†	56,921†	37,300†		
Fertility levels	2	357†	631†	224†		
Varieties × levels	8	46	127†	59†		
Seasons × varieties	16	373†	608†	687†		
Seasons × levels	8	19	19*	22		
Seasons × levels × varieties	32	24	8	11		

<sup>1</sup> Includes the 4 years, 1938-41.

\* Exceeds the 5% level of significance.

† Exceeds the 1% level of significance.

An examination of the fermentation-time test data shows that at any one location the gluten strength, as measured by the fermentation-time test, increased as the level of soil fertility increased.<sup>1</sup> This relationship exists in the weaker quality soft wheats as well as the stronger

gluten variety Michikof. These differences are very consistent and therefore highly significant. Although higher fertility of the soil definitely increases gluten strength, the greatest variation is the inherent difference between varieties. The results of the analysis indicate, also, that great differences exist between seasons, and that varieties respond differently in different seasons.

*Granulation.* Granulation tests were conducted on the wheat samples to determine the influence of soil fertility on the ease of "breaking up" or disintegration of the wheat kernel by grinding—an important milling characteristic. The data, expressed in percentage and designated as particle-size index, together with analyses of variance, are given in Table II.

The data indicate that on the Sand Field plots fertility had little, if any, influence on granulation of wheat meal. However, at the Knox County Experimental Farm and the Soils and Crops Farm, wheat samples grown on the more productive levels milled into coarser meal than those produced on the lower fertility plots. These differences, although significant, are small in magnitude. It is concluded, therefore, that granulation is a highly stable varietal characteristic, and is influenced only slightly by wide variations in soil productivity. The greatest spread in granulation is the result of variety. Purdue I, a soft wheat, shows a particle index of about 20%, whereas the hard wheat, Michikof, possesses an index of about 11%. The highly significant seasons  $\times$  varieties and seasons  $\times$  levels interactions indicate that varieties and levels responded differently in different seasons.

*Carotinoid Pigment Content.* The amount of carotinoid pigment in wheat is usually reflected by color, an important characteristic of white flour. Table III reports the amount of carotinoid pigment of wheat when grown under conditions of low, medium, and high soil fertility.

The values for total carotinoid content in wheat shown in Table III are lower than those usually reported in the literature. There is disagreement among investigators as to the material best suited for the preparation of standards in the calibration of photoelectric instruments. In this study, pure beta-carotene, which is not completely soluble in water saturated butanol, was used.

It will be noted from the data that the carotinoid content of wheat decreased as the level of fertility increased. These differences are highly significant, and the data are consistent at each of the three locations. The analysis of variance indicates that the varieties reacted uniformly in respect to carotinoid content on all levels of soil fertility. Seasons  $\times$  varieties and seasons  $\times$  levels interactions are highly significant.



TABLE II

PARTICLE-SIZE INDEX OF WHEAT MEAL AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937-41

Levels of soil fertility	Particle-size index in percent					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	22.2	19.8	18.9	18.2	11.2	18.1
Medium	21.7	18.9	18.6	18.4	11.2	17.8
High	22.8	18.5	18.4	18.2	11.3	17.8
Mean	22.2	19.1	18.7	18.3	11.2	17.9
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	19.2	19.5	18.3	17.3	10.9	17.0
Medium	18.7	19.0	17.7	16.6	10.8	16.6
High	18.6	19.2	17.4	16.5	11.3	16.6
Mean	18.8	19.2	17.8	16.8	11.0	16.7
SOILS AND CROPS FARM (LAFAYETTE) <sup>1</sup>						
Low	19.8	18.3	18.2	17.9	11.6	17.2
Medium	19.8	17.6	18.0	17.2	11.6	16.8
High	19.0	17.0	17.2	16.4	11.2	16.2
Mean	19.5	17.7	17.8	17.2	11.4	16.7
ANALYSIS OF VARIANCE						
Item	Degrees of freedom	Mean square				
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm <sup>1</sup>		
Seasons	4	135.48*	48.74*	38.24*		
Varieties	4	245.54*	167.18*	113.75*		
Fertility levels	2	0.64	1.67*	5.06*		
Varieties × levels	8	0.85	0.36*	0.25		
Seasons × varieties	16	5.61*	1.99*	1.54*		
Seasons × levels	8	8.47*	1.14*	3.87*		
Seasons × levels × varieties	32	0.65	0.09	0.20		

<sup>1</sup> Includes the 4 years, 1938-41.

\* Exceeds the 1% level of significance.

*Crude Protein.* The protein content of wheat and analyses of variance data for the fertility level experiments are given in Table IV. Wheat produced on soil of the highest fertility level at each of the three locations shows the highest percentage of protein. Plots receiving no

fertilizer produced wheat somewhat higher in protein content than those receiving only a moderate amount of plant food.

Although the five varieties used in this study are known to vary widely in their milling and baking characteristics, their range in average protein content is less than 1%. The average protein content of

TABLE III  
CAROTINOID PIGMENT CONTENT OF WHEAT MEAL AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937-41

Levels of soil fertility	Yellow pigments (expressed as carotene) in parts per million					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	1.82	1.92	1.79	1.58	1.93	1.81
Medium	1.69	1.72	1.67	1.46	1.81	1.67
High	1.64	1.70	1.61	1.42	1.72	1.62
Mean	1.72	1.78	1.69	1.48	1.82	1.70
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	1.84	2.07	2.03	1.70	2.10	1.95
Medium	1.67	1.84	1.86	1.47	1.94	1.76
High	1.65	1.79	1.78	1.46	1.87	1.71
Mean	1.72	1.90	1.89	1.54	1.97	1.80
SOILS AND CROPS FARM (LAFAYETTE) <sup>1</sup>						
Low	1.99	2.11	2.01	1.84	2.24	2.04
Medium	1.83	1.91	1.93	1.65	2.10	1.88
High	1.81	1.90	1.89	1.62	2.07	1.86
Mean	1.88	1.97	1.94	1.70	2.14	1.93
ANALYSIS OF VARIANCE						
Item	Degrees of freedom	Mean square				
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm		
Season	4	0.7853*	2.5514*	0.3414*		
Varieties	4	0.2534*	0.4469*	0.2966*		
Fertility levels	2	0.2378*	0.3937*	0.1906*		
Varieties × levels	8	0.0023	0.0030	0.0026		
Seasons × varieties	16	0.0074*	0.0134*	0.0149*		
Seasons × levels	8	0.0138*	0.0247*	0.0354*		
Seasons × levels × varieties	32	0.0012	0.0025	0.0032		

<sup>1</sup> Includes the 4 years, 1938-41.

\* Exceeds the 1% level of significance.

TABLE IV

PROTEIN CONTENT OF WHEAT AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937-41

Levels of soil fertility	Protein content in percent					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	10.02	11.55	10.39	10.89	11.11	10.79
Medium	10.20	11.08	10.17	10.66	10.97	10.61
High	11.23	12.45	11.11	11.77	12.22	11.76
Mean	10.48	11.69	10.56	11.11	11.43	11.05
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	10.14	10.96	10.22	10.56	10.98	10.57
Medium	9.74	10.62	9.98	10.26	10.52	10.22
High	10.42	11.28	10.10	10.78	11.24	10.76
Mean	10.10	10.95	10.10	10.53	10.91	10.52
SOILS AND CROPS FARM (LAFAYETTE) <sup>1</sup>						
Low	8.27	9.31	8.68	8.78	8.97	8.80
Medium	8.21	9.26	8.58	8.72	8.99	8.75
High	8.78	9.76	9.03	9.30	9.41	9.26
Mean	8.42	9.44	8.76	8.93	9.12	8.94
ANALYSIS OF VARIANCE						
Item	Degrees of freedom	Mean square				
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm <sup>1</sup>		
Season	4	23.56*	12.56*	1.39*		
Varieties	4	4.22*	2.61*	1.77*		
Fertility levels	2	9.39*	1.88*	1.54*		
Varieties × levels	8	0.10	0.08	0.01		
Seasons × varieties	16	0.21*	0.20*	0.12*		
Seasons × levels	8	2.86*	0.40*	0.06*		
Seasons × levels × varieties	32	0.06	0.04	0.01		

<sup>1</sup> Includes the 4 years, 1938-41.

\* Exceeds the 1% level of significance.

the varieties studied does not reflect their known gluten strength. American Banner, a white wheat, is known to possess the weakest quality, yet its protein content is higher than that of Purdue No. 1. Trumbull, a typical soft wheat, also shows a higher percentage of

protein, on all levels of soil fertility and at all locations, than Michikof, a hard wheat. These data indicate that protein content is of little, if any, value in appraising the gluten quality of wheats grown in the soft winter wheat area. The results shown in Table IV also substantiate the well-known fact that great differences exist among seasons

TABLE V

TEST WEIGHT OF WHEAT AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937-41

Levels of soil fertility	Test weight in pounds per bushel					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	58.2	59.0	57.5	58.9	59.6	58.6
Medium	58.4	59.0	57.3	58.8	59.6	58.6
High	58.3	58.9	57.6	58.8	59.5	58.6
Mean	58.3	59.0	57.5	58.8	59.6	58.6
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	58.5	58.4	57.0	58.5	58.7	58.2
Medium	59.5	59.0	57.6	59.2	59.6	59.0
High	59.5	59.2	57.9	59.5	59.7	59.2
Mean	59.2	58.9	57.5	59.1	59.3	58.8
SOILS AND CROPS FARM (LAFAYETTE) <sup>1</sup>						
Low	59.7	59.6	57.8	59.1	59.5	59.1
Medium	60.3	60.1	58.0	60.0	60.2	59.7
High	60.5	60.3	58.4	60.3	60.4	60.0
Mean	60.2	60.0	58.1	59.8	60.0	59.6
ANALYSIS OF VARIANCE						
Item	Degrees of freedom	Mean square				
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm		
Season	4	63.49*	24.67*	18.96*		
Varieties	4	9.42*	8.25*	9.46*		
Fertility levels	2	0.01	6.25*	3.68*		
Varieties × levels	8	0.05	0.04	0.06		
Seasons × varieties	16	1.05*	0.31*	0.35*		
Seasons × levels	8	2.78*	0.39*	0.31*		
Seasons × levels × varieties	32	0.11	0.04	0.05		

<sup>1</sup> Includes the 4 years, 1938-41.

\* Exceeds the 1% level of significance.

TABLE VI

THOUSAND-KERNEL WEIGHT OF WHEAT AND ANALYSES OF VARIANCE OF FIVE VARIETIES OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE LOCATIONS DURING THE 5-YEAR PERIOD, 1937-41

Levels of soil fertility	1000-kernel weight in grams					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	26.4	30.5	32.0	27.9	26.1	28.6
Medium	28.7	33.3	34.7	30.3	28.2	31.0
High	28.5	32.9	35.0	30.3	27.9	30.9
Mean	27.9	32.2	33.9	29.5	27.4	30.2
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	26.6	28.4	29.0	28.1	25.0	27.4
Medium	28.7	30.5	31.5	30.2	27.4	29.7
High	28.8	30.6	32.2	30.1	27.7	29.9
Mean	28.0	29.8	30.9	29.5	26.7	29.0
SOILS AND CROPS FARM (LAFAYETTE) <sup>1</sup>						
Low	31.0	33.3	35.1	31.0	28.8	31.8
Medium	32.6	35.0	36.4	33.1	30.4	33.5
High	32.3	35.0	36.8	32.9	30.7	33.5
Mean	32.0	34.4	36.1	32.3	30.0	33.0
ANALYSIS OF VARIANCE						
Item	Degrees of freedom	Mean square				
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm <sup>1</sup>		
Season	4	57.59†	59.48†	147.39†		
Varieties	4	119.66†	40.54†	68.00†		
Fertility levels	2	48.96†	46.70†	19.07†		
Varieties × levels	8	0.30	0.27	0.17		
Seasons × varieties	16	1.58†	1.24†	2.73†		
Seasons × levels	8	1.07*	2.48†	2.22†		
Seasons × levels × varieties	32	0.35	0.15	0.16		

<sup>1</sup> Includes the 4 years, 1938-41.

\* Exceeds the 5% level of significance.

† Exceeds the 1% level of significance.

and locations. Under the conditions of the experiment, all varieties reacted uniformly in protein content on all levels of soil fertility.

*Test Weight.* Test weight is an important component of wheat quality since it generally reflects flour yield. The data involving the

test weight of five varieties of wheat grown on several levels of soil fertility are shown in Table V.

On the Plainfield fine sand at Culver, Indiana, there was no relation between test weight and soil fertility. At the Knox County Experimental Farm the average test weight was increased from 58.2 lb on

TABLE VII  
ASH CONTENT OF WHEAT AND ANALYSES OF VARIANCE OF FIVE VARIETIES  
OF WHEAT GROWN ON THREE LEVELS OF SOIL FERTILITY AT THREE  
LOCATIONS DURING THE 3-YEAR PERIOD, 1939-41

Levels of soil fertility	Ash content in percent					Mean
	Purdue I	Trumbull	American Banner	Michigan Amber	Michikof	
SAND FIELD (CULVER)						
Low	2.06	1.96	1.86	1.95	1.89	1.94
Medium	1.95	1.87	1.79	1.82	1.81	1.85
High	1.94	1.91	1.80	1.80	1.78	1.85
Mean	1.98	1.91	1.82	1.86	1.83	1.88
KNOX COUNTY EXPERIMENTAL FARM (WHEATLAND)						
Low	1.76	1.68	1.69	1.67	1.67	1.69
Medium	1.76	1.68	1.70	1.70	1.66	1.70
High	1.79	1.78	1.70	1.72	1.70	1.74
Mean	1.77	1.71	1.70	1.70	1.68	1.71
SOILS AND CROPS FARM (LAFAYETTE)						
Low	1.90	1.89	1.83	1.87	1.79	1.85
Medium	1.84	1.86	1.79	1.81	1.74	1.81
High	1.86	1.86	1.77	1.81	1.79	1.82
Mean	1.86	1.87	1.80	1.83	1.77	1.83
ANALYSIS OF VARIANCE						
Item	Degrees of freedom	Mean square				
		Sand Field	Knox County Expt. Farm	Soils and Crops Farm		
Seasons	2	0.7157†	0.0488†	0.5324†		
Varieties	4	0.0436†	0.0074†	0.0160†		
Fertility levels	2	0.0464†	0.0060†	0.0092†		
Varieties × levels	8	0.0013	0.0009	0.0007		
Seasons × varieties	8	0.0036*	0.0044*	0.0013*		
Seasons × levels	4	0.0029	0.0155†	0.0007		
Seasons × levels × varieties	16	0.0010	0.0011	0.0005		

\* Exceeds the 5% level of significance.

† Exceeds the 1% level of significance.

the low-fertility to 59.2 lb on the high-fertility plots. On the Soils and Crops Farm, the average test weights for low, medium, and high levels of soil fertility were 59.1, 59.7, and 60.0 lb, respectively. Varieties varied significantly in test weight at all locations with a range of averages from 57.7 lb for American Banner to 59.6 for Michikof.

*Kernel Size.* Kernel size was determined by the weight in grams of 1000 kernels of wheat. The data were averaged according to varieties and locations and are shown in Table VI.

It will be noted that the low-fertility plots at each location produced the smallest kernels, while the wheat grown on the soils of medium and high productivity had significantly larger kernels. Significant differences were also found between seasons and between varieties.

*Ash Content.* Ash determinations were made only on the wheat samples harvested during the 3-year period, 1939-41. The data are presented in Table VII.

At the Sand Field and the Soils and Crops Farm the wheat grown on the low-fertility plots possessed a greater percentage of ash than that produced on the more productive levels. On the other hand, at the Knox County Farm, the samples from the plots receiving the heavy application of fertilizers showed the higher percentage of ash. Little difference was found in the ash content of wheat grown on the medium and high levels of fertility at all three locations. All varieties reacted uniformly in ash content on all levels of soil fertility.

### Summary

Gluten strength, granulation, carotinoid pigment, crude protein, test weight, kernel size, and ash data are reported for five varieties of wheat grown on three levels of soil fertility at each of three locations during the 5-year period of 1937-41.

The results show that soil fertility definitely influences quality in wheat. In general the quality of wheat improves as the fertility of the soil increases. Wheat produced on the well-fertilized plots was found stronger in gluten, lower in carotinoid pigments, and higher in yield of flour than that grown on the low-fertility plots. Variety, or heredity, caused the greatest variations and had the most influence in producing differences in the components of quality studied.

Since the fertility of the soil, especially in the humid region, is rapidly declining, the results would indicate that the quality of our future wheat supply in this region may be somewhat lower than that formerly obtained. However, by the addition of adequate amounts of fertilizer to the soil, wheat of satisfactory quality can be produced. Moreover, since most components of quality are hereditary, they can be amended to some extent by the breeder.

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## SIMPLIFIED PROCEDURES FOR THE DETERMINATION OF THIAMINE IN WHEAT FLOURS AND BREAD BY THE THIOCHROME METHOD

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With the introduction of the enrichment program, a growing number of laboratories have been applying an increasing amount of time to thiamine assay in wheat products. The saving of the chemist's time and of materials is always a worthy end, but it is even more imperative today that analytical procedures should require the least possible time consistent with the necessary accuracy, and that a minimum of laboratory materials be employed.

A shortened procedure for the thiochrome method was reported by Andrews and Nordgren (1941) who employed a 15-min extraction at room temperature with 25% potassium chloride in 2% acetic acid, followed by filtration and oxidation to thiochrome. The oxidation was



performed according to the method of the American Association of Cereal Chemists (Cereal Laboratory Methods, 4th ed. 1941).

Hoffer, Alcock, and Geddes (1943) have just published a modification of the procedure of Andrews and Nordgren (1941) in which the extraction is carried out at 70° for 30 min. This method, when compared with that of the A.A.C.C., was shown to give low results with unenriched commercial flours, but the difficulty could be circumvented by the use of a correction factor. For wheat and enriched flour there was no need for the correction.

Meanwhile, in England, an abbreviated procedure was independently developed by Nicholls, Booth, Kent-Jones, Amos, and Ward (1942) and by Booth (1942) for the estimation of thiamine in the national wheat meal. They omitted hot extraction, enzyme digestion, and base exchange purification. However, their procedure differed from that of the above American workers in the use of mineral acid without salt for the extraction and in the introduction of methanol prior to oxidation, as recommended by Harris and Wang (1941) and Wang and Harris (1942) for the stabilization of thiochrome.<sup>1</sup> A comparison of this method, with a few minor modifications, with the collaborative method of Hennessy (1942), changed in only one detail, was undertaken for wheat flours and bread. The Hennessy method was chosen for the comparison since, in the opinion of the author, it is the best of the American methods employing the adsorption step. Consideration was also given to the question of the stability of standard solutions of thiamine.

### Experimental

#### *Simplified Procedure for Patent, Straight, Clear, Low Grade, and Enriched Flours.*

A guide to the proportion of extracting acid to sample weight has been given by Hennessy (1942). We have found it convenient to employ 50 ml of 0.1*N* H<sub>2</sub>SO<sub>4</sub> to 10 g of patent or straight, 4 g of enriched patent or straight, 8 g of clear, or 2 g of low grade flour.

Add the sample to the acid in a graduate with a ground glass mouth, stopper, and place in a mechanical rocker or slow shaker for 30 min or shake by hand at least once every 5 min. Filter through a paper having the porosity of No. 4 Whatman, fluted to increase the filtration rate, and after the filtrate begins to come through clear, pour back the first turbid liquid for a second filtration. In each of two separatory centrifuge tubes (E. Machlett & Sons) mix 5 ml of extract with 5 ml CH<sub>3</sub>OH. To one tube add rapidly 3 ml of 15% NaOH, from a pipette with a large orifice, followed immediately by 2 drops of 1% K<sub>3</sub>Fe(CN)<sub>6</sub>; mix at once, and after 45–60 seconds add 20 ml of isobutanol previously saturated with distilled water. Repeat with the other tube but omit the K<sub>3</sub>Fe(CN)<sub>6</sub>. Stopper and shake tubes well for about 60 seconds. Centrifuge at 500–600 rpm for 30–45 seconds and drain off the bottom layer. To remove the water still suspended in the isobutanol solution, filter

<sup>1</sup> Since this manuscript was submitted, a report has been published by the Vitamin B<sub>1</sub> Subcommittee of the Accessory Food Factors Committee of the Medical Research Council and the Lister Institute, giving the results of comparative tests for thiamine by various methods (Biochem. J. 37: 433–439. 1943).

through No. 4 Whatman or similar grade of fast paper. Pass the filtrate through a fresh paper in a clean funnel directly into the tube or cuvette employed with the fluorometer. Measure the fluorescence of the unknown ( $X$ ) and the blank ( $B_x$ ) after adjusting the instrument to maximum galvanometer scale reading with a standard quinine sulfate solution (for the Pfaltz and Bauer instrument we use 0.5  $\mu\text{g}$  per ml in 0.1*N*  $\text{H}_2\text{SO}_4$ ). Standardization with a thiamine solution of known concentration is effected by placing 5 ml of a standard solution of thiamine hydrochloride in 0.1*N*  $\text{H}_2\text{SO}_4$  (0.4  $\mu\text{g}$  of thiamine per ml) into each of two separatory centrifuge tubes and treating in the same manner as the extract to obtain the fluorescence of the standard ( $S$ ) and the blank ( $B_s$ ).

The data are used to calculate the concentration of thiamine in the sample as follows:

$$\mu\text{g thiamine per g flour} = \frac{X - B_x}{S - B_s} \cdot \frac{50}{W} \cdot \frac{2}{3}$$

where  $W$  = weight of sample in grams.

*Procedure for Whole Wheat Flour.* Since whole wheat contains significant concentrations of interfering substances that decrease fluorescence, it is necessary to eliminate their effect. This may be accomplished by measurement of the fluorescence produced by the extract in the presence and absence of added thiamine as follows:

To 50 ml of 2%  $\text{HCl}$  (wt./vol.) in a graduate, add 4 g of the sample, stopper, shake, and allow to stand overnight at room temperature. In a separate graduate, repeat in a parallel manner with 2%  $\text{HCl}$  containing 8  $\mu\text{g}$  of thiamine hydrochloride (2  $\mu\text{g}/\text{g}$  sample). In the morning, filter as in the preceding section. Use 5 ml of filtrate in each case for the oxidation to thiochrome, following the procedure outlined previously except that 2 drops of 3%  $\text{K}_3\text{Fe}(\text{CN})_6$  are used. Measure the fluorescence produced by the extract ( $X$ ), and by the extract containing the added thiamine ( $S$ ). Blank determinations are carried out by omitting the  $\text{K}_3\text{Fe}(\text{CN})_6$  as previously described. The calculation is:

$$\mu\text{g thiamine per g sample} = (X - B_x) \left( \frac{2}{(S - B_s) - (X - B_x)} \right)$$

Since  $B_x$  and  $B_s$  in this case are essentially equal,

$$\mu\text{g per g} = \frac{X - B_x}{S - X} \cdot 2$$

#### *Procedure for Bread.*

To 50 ml of acetate buffer having a pH of 4.0 (36 ml of 0.1*M*  $\text{CH}_3\text{COONa}$  + 164 ml of 0.1*M*  $\text{CH}_3\text{COOH}$ ) add 8 g of sample and 0.2 g of takadiastase. Another vessel is prepared in the same way except that the acetate buffer contains 16  $\mu\text{g}$  of thiamine hydrochloride. After shaking well, the vessels are kept at 40°C overnight. The filtration of the extract and subsequent steps follow exactly the procedure given for whole wheat, and the calculation is identical.

### Comparison of Methods

The flours used to obtain the comparative data in Table I were milled from a wide variety of spring and winter wheats grown in various localities, and the data demonstrate the satisfactory agreement between assays made according to the procedures which have just been described and a slight modification of the collaborative method of Hennessy (1942). The departures from the Hennessy method consisted of adding the alkali before the ferricyanide in the oxidation step,

TABLE I  
COMPARISON OF THIAMINE METHODS

Sample	Hennessy method <sup>1</sup>	Present short method
	$\mu\text{g/g}$	$\mu\text{g/g}$
<i>Flours</i>		
Enriched patents	4.40 4.58 4.78 4.58 4.67	4.40 4.54 4.76 4.58 4.62
Patent	0.56 0.59 0.38 0.38 0.37	0.55 0.59 0.38 0.38 0.38
Straight grade	0.80 0.95 1.10 0.58 0.89	0.80 0.94 1.09 0.62 0.83
Clear	2.49 2.04 2.20 2.11 1.40	2.40 2.05 2.35 2.15 1.30
Low grade	7.07 6.99 9.25 5.67 2.83	6.73 7.36 9.44 5.39 2.93
Whole wheat	5.00 5.30 3.86 4.75 3.82	5.07 5.33 3.76 4.75 4.07
<i>Bread</i>		
White bread <sup>2</sup>	2.28 2.28 2.16 2.55 2.64	2.42 2.27 1.98 2.64 2.46

<sup>1</sup> These data have been corrected for the volume of the sample in the extracting medium; furthermore the alkali was added before the ferricyanide in these determinations.

<sup>2</sup> Values converted to 38% moisture basis.

and in correcting the results for the volume of the sample in the extracting medium. In the published method no such correction is made, and consequently the results are always too high. For samples under 4 g the magnitude of the correction is small and usually may be neglected, but it was found that with a 10-g sample an error of about 7.0% is involved and a 5-g sample gave an error of about 3.5%. This

was shown in the following manner: The 75 ml of acid plus sample plus 5 ml of acetate-enzyme solution required 13 ml of water in the case of a 10-g sample, a total of 93 ml of liquid to make up the volume to the 100-ml mark on a volumetric flask; for a 5-g sample the total volume of liquid added was 96.5 ml. However, the calculation is based on the presence of 100 ml of liquid which is obviously not there. It would be simpler, of course, to employ a fixed volume of liquid and thus obviate the need for a correction. This is the procedure followed in both the British and regular A.A.C.C. methods.

### Notes

There are a number of points concerning the foregoing procedures that deserve mention:

The principle of standardizing a determination by reference to a parallel measurement on an equivalent sample to which a known quantity of thiamine has been added is the soundest to employ. In this manner, all of the interfering influences that might be present exert equal effects on both the determination and the standardization and thus are cancelled. Since each sample must be standardized separately, this method involves more measurements than in the usual procedure of applying one control estimation on a standard thiamine solution to all of the determinations made at one time. For those flours which are relatively free from interfering concomitant materials, the latter method can be safely employed, but for whole wheat flours or bread this is not the case; hence the difference in the standardization procedure given for the latter compared to that for the patents, clears, etc. For instance, a whole wheat flour giving a value of  $5.07 \mu\text{g/g}$  by the procedure described in the present paper gave only  $3.74 \mu\text{g/g}$  when standardized against a pure thiamine solution subjected to the same treatment.

It is of interest to note that the volume of extracting acid does not enter into the calculation for the whole wheat and bread methods. Provided the concentration of thiamine in the extract is in the proper range, the actual volume of acid used will be without influence as long as an equal volume is employed in the standardization.

In those instances where the 30-min extraction period is used, the subsequent filtration requires an additional 30 min or so, depending on the nature of the flour and the size of the sample. The true extraction period is thus greater than 30 min. With more coarsely ground material, the extraction time would naturally have to be longer, and that is the basis for the overnight extraction of the whole wheat flour. This period is probably greater than necessary but was employed for convenience. A sample giving a thiamine content of  $5.07 \mu\text{g/g}$ ,

when subjected to overnight extraction, showed only  $3.72 \mu\text{g/g}$  when extracted for 30 min.

With flours which are milled from wheat mixes containing appreciable percentages of Durum wheat, a serious interfering fluorescence can be eliminated, as recommended by Harris and Wang (1941) and Booth (1942), by first shaking the acid extract with isobutanol, separating, and discarding the isobutanol layer before adding the methanol and reagents for oxidation.

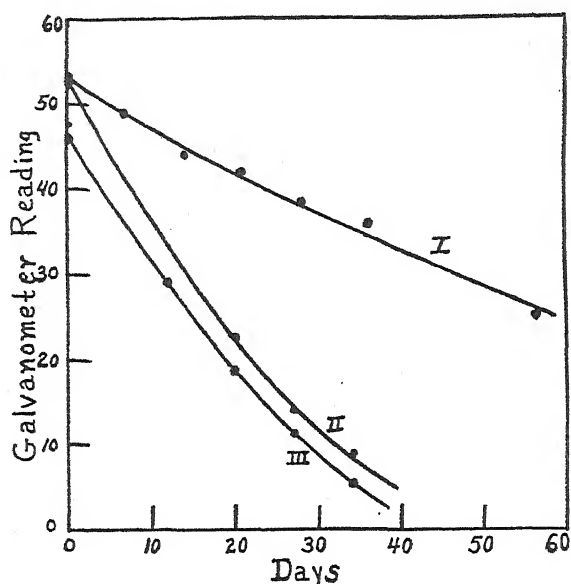


Fig. 1. Stability of standard thiamine solutions ( $0.4 \mu\text{g/ml}$ ) stored in the dark at  $6^\circ$ . I. In  $0.1N \text{ H}_2\text{SO}_4$  (5 ml used with 5 ml  $\text{CH}_3\text{OH}$  prior to oxidation); II. In a solution of equal volumes of  $0.1N \text{ H}_2\text{SO}_4$  and  $\text{CH}_3\text{OH}$  (5 ml used with 5 ml,  $\text{CH}_3\text{OH} - 0.1N \text{ H}_2\text{SO}_4$ , 1 : 1); III. In absolute  $\text{CH}_3\text{OH}$  (5 ml used with 5 ml  $0.1N \text{ H}_2\text{SO}_4$ ).

Wang and Harris (1942) have indicated the value of adding the alkali before the ferricyanide in the oxidation step. In the procedures of Hennessy (1942) and Hoffer *et al* (1943) the two are added together, and in the A.A.C.C. method they are added in the reverse order.

The amount of ferricyanide required will depend on the nature and quantity of the oxidizable impurities present in the extract. Wang and Harris (1942) have indicated that the proper amount is the least necessary to maintain the yellow ferricyanide color for over 30 seconds.

The use of either filtration through paper or addition of ethanol to clarify the isobutanol solution of thiochrome (Nicholls *et al*, 1942) is simpler, in the author's opinion, than the use of anhydrous sodium sulfate. The advantage of filtration over the use of ethanol for clarifi-

cation is that particles that may be suspended in the liquid are removed. We have found it unnecessary to subject Whatman No. 4 paper to preliminary extraction with isobutanol although the British workers have found this treatment advisable to remove fluorescent materials from the paper they employed.

The larger volume of isobutanol was employed to save time by discarding the last few ml in each of the two filtrations prior to the measurement of fluorescence.

### Stability of Standard Thiamine Solutions

The effect of methanol on the stability of standard thiamine solutions stored in the dark at 6°C is apparent from Figure 1. While methanol stabilizes thiochrome or its formation from thiamine, as shown by Harris and Wang (1941), it increases the rate of gradual decomposition of stored thiamine solutions. Results shown in Figure 1 also indicate that it is unsafe to use a standard solution of thiamine in dilute acid after it has been stored for more than a few days.

### Summary

Comparisons have been made between British procedures for the thiochrome determination of thiamine, with minor modifications, and the collaborative method of Hennessy, changed in one detail, when applied to wheat flours and bread. The two methods give results which are in satisfactory agreement, provided that a correction is applied to the latter to compensate for the volume of the sample in the extracting liquid.

Details have been reported of procedures for wheat flours that do not require extraction at elevated temperatures, enzyme digestion, or base exchange purification; and that eliminate the hot extraction and base exchange step in the case of bread. These methods are essentially the same as those of the British, and they offer certain advantages over the procedures generally used in America. Discussion of certain of the steps in the methodology has been included.

It was shown that methanol increases the rate of destruction of thiamine in solution.

### Acknowledgment

The author wishes to express his appreciation to Betty Sullivan and Marjorie A. Howe for their helpful suggestions.

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## FACTORS THAT INFLUENCE THE PHYSICAL AND OTHER PROPERTIES OF WHEAT. V. EFFECT OF FREQUENT RAINS ACCOMPANIED BY STORMS ON BLACKHULL, CHIEFKAN, AND TENMARQ<sup>1</sup>

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Extensive investigations of the effects of weathering on the quality of wheat have been made by the author (1936, 1941, 1942, 1943, 1943a, 1943b, and with Johnson, 1943), by Bracken and Bailey (1928), Whitcomb and Johnson (1928), and by Larmour, Malloch, and Geddes (1933). The results of various minor studies have also been briefly described in the annual reports of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, for the years 1928, 1931 to 1935, 1941, and 1942. The general conclusion has been that effects of weathering on wheat, provided that no germination has occurred, are by no means as serious as indicated by a superficial visual examination of the samples.

Experimental investigations under natural conditions are difficult to make because they must be confined to seasons when the weather is suitable. This condition occurred during 1942 in Kansas where heavy rains occurred during the late part of the growing season and at harvest time. The results of tests on samples collected from the experimental fields at Manhattan and elsewhere throughout Kansas are reported in this paper.

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<sup>1</sup> Contribution No. 102, Department of Milling Industry.

### Weather Conditions Prevailing in 1942

The harvest season of 1942 had abnormally heavy rains accompanied by storms during the greater part of June, both during ripening of wheat and the time it was mature for cutting. July had considerably less than half the normal rainfall for that month, while the August rainfall was nearer normal. Detailed rainfall during June, July, and August at Manhattan, Kansas, is given in Table I. The 12.72 inches

TABLE I  
RAINFALL AT MANHATTAN, KANSAS, FOR JUNE, JULY, AUGUST, 1942

Date	June	July	Aug.	Date	June	July	Aug.
	<i>inches</i>	<i>inches</i>	<i>inches</i>		<i>inches</i>	<i>inches</i>	<i>inches</i>
1	2.24 <sup>1</sup>			17	0.45		
2				18	1.20		
3		0.46	0.51	19	3.02	0.01	
4			0.06	20	1.77	0.04	
5	0.02	0.14		21		0.07	
6			0.10	22			
7	0.31		0.14	23			
8	0.01	0.48	0.02	24	1.80	0.06	
9	0.09		0.32	25		0.21	0.18
10	0.30			26			1.20
11	0.05		0.06	27			
12	0.45			28			
13	0.04		0.28	29	0.82		
14			0.40	30			
15	0.15			31			
16				Total	12.72	1.47	3.27

<sup>1</sup> On May 31 there was a rainfall of 0.46 inch.

rainfall in June at Manhattan was nearly three times normal. During the frequent rains in the middle of June, several storms occurred, causing first lodging and then twisting. This made gathering of samples difficult. Lodging occurred earlier in spots, but finally all the wheat was lodged or the straws broken and tangled.

Weather conditions during the harvest of 1942 at Manhattan were much different from the harvest of 1941 when only five light rains, totaling 0.98 inch, fell during the period from June 10 to July 22, and there were no storms in 1941. The results of the 1941 harvesting studies have been reported by Swanson (1943b).

### General Plan of Investigations

*Materials.* Three varieties were included in the 1942 experiments—Blackhull, Chiefkan, and Tenmarq. These had been grown on plots in the field used for variety testing by the Department of Agronomy.

*Plan of Studies.* There were five parts to these studies: (1) changes in the wheat kernels during maturing or ripening from the early-milk



stage of the kernels to the hard-dough stage or maturity, and also the effects of drying in sun and shade; (2) effects of exposure in the field for about 4 weeks after maturity; (3) effects of exposing small shocks of wheat cut at the hard-dough stage; (4) effects of soaking wheat in the straw and wetting as grain; (5) observations on samples of the three varieties grown in various parts of Kansas.<sup>2</sup>

*Laboratory Tests.* The effects of the various factors on the wheat grain were measured by test weight, internal texture counts, and by milling and baking the samples included under parts 2 and 3 of the plan. Supplementary tests were also made for moisture content of wheat, and for protein, ash, and moisture content of flours used for baking. The samples cut before the hard-dough stage were scoured in a barley pearler.

### Procedure and Results of Physical Tests on Manhattan Samples Samples Cut before the Hard-Dough Stage

*Procedure.* First cutting of small samples before the hard-dough stage was on June 12. Cuttings were then made on June 15, 17, and 19. The weather was cool, cloudy and, as can be seen from Table I, rains were of almost daily occurrence up to June 20 when the wheat was approaching hard-dough stage. No brown color had appeared on the kernels harvested on June 12 and 15, but on June 17 the kernels were turning brown, and on June 19 they were nearly all brown and the interior was at the hard-dough stage. All kernels harvested before the hard-dough stage became dark brown and hard on drying. Three small bundles of each variety were cut on each date. One was used for determining the moisture content of the wheat kernels, another was placed to dry out of doors, and the remaining bundle was dried in the wheat nursery shed. The study of the effects of drying out of doors and inside was only partially satisfactory because of the prevalence of cool, cloudy weather.

The bundles used for the moisture determination of the grains were threshed on the small nursery thresher, and weighed samples of the grain were exposed to the air in shallow pans in the laboratory to obtain data for the amount of moisture lost in air drying. From the additional moisture contents subsequently obtained by the usual oven treatment of the air-dry samples, the total moisture contents at the time of cutting were calculated.

The bundles dried out of doors and bundles dried under cover were later threshed on the nursery thresher. The following determinations were made on the samples: test weight (micro method, Swanson, 1942),

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<sup>2</sup> These samples were made available by Dr. H. H. Laude, of the Department of Agronomy.

internal texture counts, and effects of miniature scouring on test weight. This scouring was done by passing 100-g samples through a barley pearler with the outlet gate open. Several preliminary tests had shown that this treatment was closely equivalent to scouring. The internal textures were determined on kernel sections made with a barley cutter. The data from these various measurements on wheat cut before the hard-dough stage are given in Table II.

TABLE II  
MOISTURE, TEST WEIGHT, AND INTERNAL TEXTURE COUNTS OF WHEAT  
SAMPLES CUT BEFORE THE HARD-DOUGH STAGE

Date cut June	Moisture average all var.	Test weight								
		Blackhull			Chiefkan			Tenmarq		
		Shade	Sun	After scour- ing	Shade	Sun	After scour- ing	Shade	Sun	After scour- ing
	%	lb/bu	lb/bu	lb/bu	lb/bu	lb/bu	lb/bu	lb/bu	lb/bu	lb/bu
12	49	55.6	55.2	58.4	58.5	57.2	61.1	54.8	56.5	57.9
15	48	56.4	55.5	59.6	59.9	57.7	63.6	55.8	54.3	58.5
17	47	56.9	55.1	60.1	59.1	59.2	61.3	57.2	57.8	60.2
19	44	56.6	55.7	59.5	60.8	59.7	62.9	59.0	58.1	61.4
Internal texture counts (Average for sun- and shade-dried samples)										
		Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy
	%	%	%	%	%	%	%	%	%	%
12	49	55	35	10	94	4	2	85	12	3
15	48	65	27	8	98	2	0	93	6	1
17	47	69	24	7	97	2	1	88	7	5
19	44	64	28	8	97	2	1	76	17	7

*Results.* A comparison of the results for the samples cut June 12 with those cut on June 19 shows that Blackhull had made the least increase in test weight, Tenmarq the greatest increase, and Chiefkan intermediate. The comparatively small increases in test weight between June 12 and June 19 were probably due to the sometimes cloudy and rainy weather. All samples, except two of Tenmarq, decreased in test weight from drying out of doors because of shriveling. Drying out of doors would no doubt have had greater effect in hot, dry weather. The two exceptions of Tenmarq were probably due to variations in field sampling.

The test weights after scouring were in all cases higher than before this treatment, showing that the comparative looseness of the outer bran, even of immature grain, has a large influence on test weights.

While this method of scouring may not be very accurate, the figures are comparable and indicate that this treatment had the greatest effect on Blackhull and the least on Tenmarq with Chiefkan intermediate.

As to internal texture condition, Blackhull showed the lowest vitreous and highest mealy counts; Chiefkan showed the highest vitreous and lowest mealy counts; Tenmarq was intermediate. There was no consistent trend between the first and succeeding cuttings.

### **Samples Cut in and after Hard-Dough Stage and Exposed in Shocks**

*Procedure.* The first cutting in this series was made on June 22, when the grain was in the hard-dough to hard stage. The weather at this time was still cool for the season. On this date and at each subsequent cutting, three bundles were gathered in order to have enough grain for milling and flour for baking. Besides these three bundles, a small sample was cut from each variety for the determination of moisture, proceeding in the same manner as with the samples cut earlier.

The wheat cut on June 22 appeared as mature as is usual when cutting is started with a binder for drying in shocks. Because of the rains and storms, the wheat was not only lodged but tangled. This made gathering difficult and the field losses increased progressively with the later cuttings. All bundles were placed in the shed soon after gathering and threshed when dry.

The bundles for exposure in the shocks were cut June 25-27 when the moisture content of the grain was about 20%. The small shocks were tied to wooden stakes and were not covered except with screen wire to keep off the birds. There were five small shocks of each variety, four of which were threshed at weekly intervals, but the last one was exposed until September 2, over 2 months. Hence, it was exposed to the heavier rains in August.

*Results.* Test weights were taken by the official method and milling was done on the Buhler mill. The flours were analyzed for moisture, protein, and ash. The figures for moisture and protein were used to calculate the absorption for making the mixograms (Swanson and Johnson, 1943), and also for baking. The dates of cutting the wheat and threshing the shocks, the moisture percentages of the grain at the time of cutting, the test weights, the flour yields, and ash are all given in Table III.

Highest test weights were obtained on the samples cut on June 22. After this there was a gradual decrease which was related to the time of exposure in the field. The total decrease from exposure in the field was 2.3 lb for Blackhull, 3.0 for Chiefkan, and 2.3 for Tenmarq. While Chiefkan had a higher test weight throughout than either of

TABLE III

TEST WEIGHT, FLOUR YIELD, AND FLOUR ASH CONTENT FOR WHEAT SAMPLES CUT IN AND AFTER HARD-DOUGH STAGE AND AFTER EXPOSURE IN THE FIELD OR IN SHOCKS

Date cut	Moisture when cut	Test weights			Flour yield and flour ash					
		Black-hull	Chiefkan	Tenmarq	Blackhull		Chiefkan		Tenmarq	
					Yield	Ash	Yield	Ash	Yield	Ash
EXPOSED IN THE FIELD										
June 22	%	lb	lb	lb	%	%	%	%	%	%
26	37	57.6	60.7	58.0	72.0	0.47	72.0	0.46	71.0	0.45
July 1	20	56.4	59.9	53.7	71.0	0.46	73.0	0.47	72.6	0.50
6	13	57.0	59.1	56.2	71.0	0.46	73.0	0.45	71.1	0.43
13	14	56.3	58.4	55.8	74.0	0.46	74.0	0.49	73.2	0.45
20	11	56.7	58.2	55.8	70.9	0.44	76.0	0.46	73.4	0.46
22	12	56.0	58.0	54.6	72.8	0.41	73.7	0.45	72.2	0.44
	12	55.3	57.7	55.7	72.0	0.44	72.0	0.48	72.0	0.45
EXPOSED IN SHOCKS										
Date threshed	Moisture when threshed									
July 1	%	lb	lb	lb	%	%	%	%	%	%
6	12	56.5	59.4	52.6	70.2	0.46	72.5	0.51	71.0	0.41
13	11	55.5	59.2	56.6	70.2	0.45	72.0	0.47	71.4	0.45
20	10	54.4	59.9	55.5	70.1	0.46	71.0	0.47	73.6	0.48
Sept. 2	10	55.8	59.5	55.6	72.7	0.47	72.0	0.46	71.9	0.45
	13	54.8	57.6	54.6	73.0	0.44	73.5	0.46	71.3	0.45

the other two, the decrease in the test weight of Chiefkan was somewhat greater.

Exposure in the shocks caused notably less change in test weight than exposure in the field. The few rains in July caused no consistent change in test weight, but the exposure to the heavier rains in August decreased the test weight of Blackhull and Tenmarq 1 lb and of Chiefkan 1.9 lb below the sample threshed July 20.

The flour yields <sup>3</sup> did not decrease correspondingly with the lowering of test weight, thus confirming previous investigations (Swanson, 1941, 1943b). The extraction percentages correspond to that obtained for straight flour and the ash figures indicate fairly uniform milling. The flour yields for Blackhull were significantly lower than for either Tenmarq or Chiefkan.

The changes in internal texture of the samples from the field exposure and the shock exposure are given in Table IV. The 0.82-inch

<sup>3</sup> Credit is due Warren F. Keller, Research Miller, for making the milling tests.

TABLE IV  
CHANGES IN INTERNAL TEXTURE OF WHEAT FROM FIELD AND SHOCK EXPOSURE

Date cut	Blackhull			Chiefkan			Tenmarq		
	Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy
SAMPLES FROM FIELD EXPOSURE									
	%	%	%	%	%	%	%	%	%
June 22	55	30	15	98	1	1	93	5	2
26	50	36	14	98	1	1	92	5	3
July 1	44	38	18	94	6	0	62	36	2
6	25	52	23	85	8	7	21	50	29
13	12	49	39	89	9	2	27	50	23
20	5	49	46	73	26	1	25	53	22
22	17	45	38	62	27	11	14	69	17
SAMPLES FROM SHOCK EXPOSURE									
Date threshed	%	%	%	%	%	%	%	%	%
July 1	51	39	10	100	0	0	41	41	18
6	57	29	14	98	1	1	85	11	4
13	56	35	9	94	2	4	70	24	6
20	47	32	21	96	3	1	63	28	9
Sept. 2	18	46	36	87	7	6	38	25	17

rain on June 29 and the 0.46-inch rain on July 3 (Table I) seem to have produced marked changes in the internal texture since there is a notable increase in the mealy condition between the samples obtained on June 26 and July 1 and much more between the samples obtained on July 1 and July 6. Blackhull showed the greatest change and Chiefkan the least with Tenmarq intermediate. Chiefkan exhibited a remarkable resistance to exposure to rains. There were no extensive internal changes in the samples exposed in the shocks except the ones threshed September 2, indicating that, although the shocks were not covered, the compact massing of the wheat heads afforded a fairly good protection against the rains. Only the shocks left out of doors until September 2 had the dark gray appearance associated with prolonged exposure.

A comparison of the flour yields in Table III with the internal textures in Table IV shows that changes from vitreous to mealy interiors were not reflected in decreased flour yields.

### Effects of Wetting Grain before and after Threshing

Observations made in previous experiments indicated that wetting wheat in the head did not produce as great changes as when water was added to the threshed grain. The glumes seemed to afford a considerable protection against wetting.

*Procedure.* Two bundles of each of the three varieties—Blackhull, Chiefkan, and Tenmarq—were cut on June 22 and again on June 26, when the wheat was hard and had not yet been affected by wetting by rain as shown in Table IV. After drying in the shed for a few days, one bundle from each pair was soaked for 3 hours, heads down, in a large can. This was done toward evening in order to prolong the period of wetness. The three bundles so wetted were then exposed until dry, but were protected from further wetting by rain, after which they were threshed.

The other bundle from each pair was threshed as soon as dry and the grain divided into two portions. One was used as a check, not wetted; the other portion was wetted by soaking the wheat in water for  $2\frac{1}{2}$  hours and then dried. The grain was intentionally soaked a somewhat shorter time than the heads. When again dry these three portions of grain were then tested for test weight (micro method) and internal textures.

TABLE V  
EFFECT OF WETTING THRESHED AND UNTHRESHED WHEAT ON TEST  
WEIGHT AND INTERNAL TEXTURE OF THE GRAIN

Date cut	Variety	Treatment	Test weight	Internal texture		
				Vit.	Semi vit.	Mealy
June 22	Blackhull	Not wetted	<i>lb/bu</i> 59.9	% 58	% 33	% 9
		Wetted in heads	56.1	46	41	13
		Wetted as grain	56.5	0	34	66
	Chiefkan	Not wetted	59.0	93	4	3
		Wetted in heads	59.5	94	3	3
		Wetted as grain	55.3	62	32	6
	Tenmarq	Not wetted	58.5	90	6	4
		Wetted in heads	56.5	58	36	6
		Wetted as grain	53.9	5	50	45
June 26	Blackhull	Not wetted	56.8	59	23	18
		Wetted in heads	56.5	38	39	23
		Wetted as grain	53.7	8	28	64
	Chiefkan	Not wetted	60.4	98	2	0
		Wetted in heads	59.2	94	5	1
		Wetted as grain	57.2	67	26	7
	Tenmarq	Not wetted	56.4	69	23	8
		Wetted in heads	54.9	61	32	7
		Wetted as grain	53.1	15	39	46

*Results.* The data in Table V show that wetting by soaking the heads in water notably decreased the test weight, but not as much as

by soaking the grain. The internal textures were also notably changed by soaking the heads but not nearly as much as by soaking the grain. In a previous experiment (Swanson, 1936) it was observed that soaking for 10 to 30 minutes had comparatively little effect. The artificial sprinkling of standing wheat produced no measurable effects. It is thought that the rains which fall as drizzles and at night have a much greater effect than small rains in the day time.

### Results of Protein, Mixing, and Baking Tests on Manhattan Samples

*Method.* The flours from the wheat samples represented by the data in Tables II and IV were baked <sup>4</sup> using the formula: flour 100 g,

TABLE VI

PROTEIN CONTENT, MIXING TIME, AND LOAF VOLUME FOR FLOURS MILLED FROM WHEATS CUT AT THE HARD-DOUGH STAGE AND AFTER EXPOSURE TO RAINS

Date cut	Rain-fall after cutting	Blackhull			Chieffkan			Tenmarq		
		Pro-tein <sup>1</sup>	Mixing time	Loaf volume	Pro-tein <sup>1</sup>	Mixing time	Loaf volume	Pro-tein <sup>1</sup>	Mixing time	Loaf volume
EXPOSED IN THE FIELD										
	<i>inches</i>	<i>%</i>	<i>min</i>	<i>cc</i>	<i>%</i>	<i>min</i>	<i>cc</i>	<i>%</i>	<i>min</i>	<i>cc</i>
June 22	0.0	14.2	2.2	1043	15.0	1.6	860	13.7	3.1	1025
26	1.80	14.9	2.1	1060	14.9	1.6	843	15.0	3.1	1170
July 1	2.62	14.8	2.2	1023	15.2	1.7	800	14.2	3.0	1043
6	3.22	14.5	2.4	1020	15.0	1.8	870	13.5	3.1	958
13	3.70	14.1	2.2	983	15.4	1.8	840	14.3	3.3	1038
20	3.75	14.4	2.4	1008	14.5	1.8	765	14.4	3.4	1040
22	3.82	14.5	2.7	1080	14.7	1.8	825	13.9	3.6	980
EXPOSED IN SHOCKS										
Date threshed	Rain-fall after threshing									
	<i>inches</i>	<i>%</i>	<i>min</i>	<i>cc</i>	<i>%</i>	<i>min</i>	<i>cc</i>	<i>%</i>	<i>min</i>	<i>cc</i>
July 1	0.0	15.2	2.2	1025	14.8	1.5	750	13.6	3.6	1010
6	0.60	14.8	2.2	1010	14.8	1.6	758	14.3	3.1	1058
13	1.08	15.2	2.2	1035	14.7	1.8	765	14.9	3.4	1055
20	1.13	13.7	2.2	950	14.9	1.6	785	14.6	3.6	1040
Sept. 2	3.61	15.0	2.4	1095	15.0	1.9	908	14.4	3.6	1028

<sup>1</sup> Protein content ( $N \times 5.7$ ) is expressed on "as is" moisture basis.

dry milk solids 4 g, shortening 3 g, sugar 6 g, yeast 2 g, salt 1.5 g,  $KBrO_3$  3 mg, and water, calculated on the basis of protein and moisture. This "rich" formula had been shown in previous experiments (Swanson, 1943) to be best when observations are being made to discover

<sup>4</sup> Credit is due to John A. Johnson, Assistant Baking Technologist, for performing the baking tests.

any differentiation among samples exposed to varying amounts of weathering. The mixing time was calculated from the characteristics of the mixograms and a factor correlating with the bakery mixer.

*Results.* The loaf volume, mixing time, and protein percentages of the flours are given in Table VI. The data on grain texture and crumb color are not included since they did not seem to add any important information.

It is very evident that the loaf volumes vary most between the varieties and very little according to exposure. That is, the exposure in the field and shocks did not have any considerable effect on the baking value. The mixing time was slightly longer for the samples exposed the most; in previous investigations (Swanson, 1943b) it was found that the mixing times of the weathered samples were distinctly longer than the nonweathered.

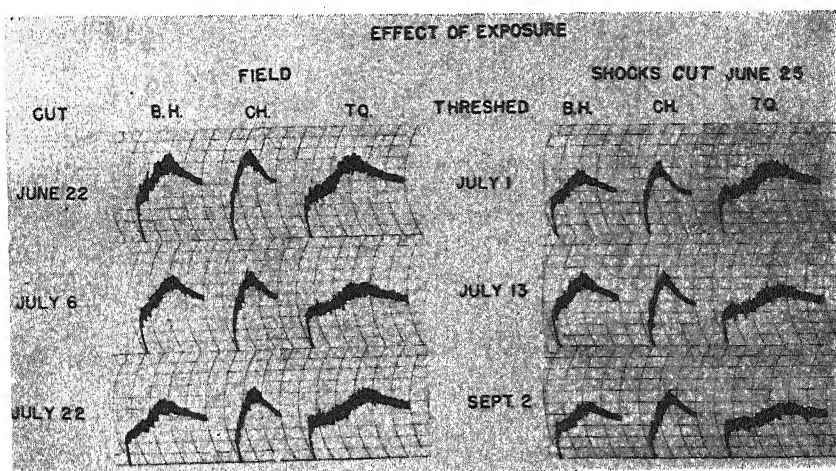


Fig. 1. Mixogram patterns for flours milled from wheat subjected to exposure in the field and in shocks.

The effects of exposure on selected mixograms are shown in Figure 1. It will be observed that some changes occur in the shapes of the curves with changes in the dates of cutting and threshing. It will be noted that the curves for Chiefkan show less change with time of exposure than do those for Blackhull and Tenmarq.

#### Samples from Experimental Fields in Various Parts of Kansas

The unusually wet weather which prevailed at Manhattan in June (Table I) was also experienced in the main wheat belt of Kansas. This presented an opportunity to observe the effects on these same three varieties of frequent rains between heading and harvesting in various locations.



TABLE VII

RAINFALL IN INCHES DURING KERNEL FORMATION AT VARIOUS LOCATIONS IN KANSAS

Date	Colby	Columbus	Dodge	Garden City	Hays	Hutchinson	Kingman	Meade	Thayer	Tribune
May 21										
22										
23						— <sup>1</sup>				
24										
25			—				—			
26										
27								—		
28		—			—					—
29										
30				—						
31			0.06	0.16	4.14		0.24	0.90		
June 1					0.25	0.50				
2	—									
3										
4	T		0.40	0.02				0.03		
5	T			T	0.04	2.00			—	
6			0.36						2.31	T
7	0.02	1.83	0.15	0.33		1.07	0.66	0.63	1.73	0.14
8	1.93	0.43	0.61	1.13	0.32	0.18	0.23	0.25	0.38	0.53
9	0.40	1.25		0.01	0.74	0.10	0.06	2.05	0.64	2.51
10		0.73				0.03		0.11		
11		0.63								
12	0.05			0.15	0.41	0.75	0.40	0.06	0.83	
13	0.62	0.97			0.15					0.72
14				0.18	T		0.09			
15		0.42			0.22	0.13			0.68	0.15
16	0.07			T	0.39	1.17	0.27			
17	0.02	0.49	0.84	0.22	2.37	1.78	0.54		1.21	
18	0.59			0.06	0.91	0.79		1.88	1.73	0.03
19		1.09			T	0.51	1.21		0.73	T
20	0.05	= <sup>1</sup>		0.13					1.05	
21	0.10		0.56	0.90	0.08	0.90	1.03		1.70	0.16
22	0.30		=	0.01	0.33	0.40				1.11
23	0.02			0.03	T				0.33	0.10
24	T	0.62								0.06
25	0.02									
26	T									
27				T	0.79	=				
28				0.17	0.09	0.61				
29						0.54	2.01	=		0.55
30				=	=				=	0.12
July 1	T			0.03						0.07
2				0.09						0.02
3	=									
4							=			
5										=
Total	4.19	7.84	2.98	3.50	11.45	10.31	6.74	5.91	13.32	6.27

<sup>1</sup> Single line represents date of heading and double line date of cutting.

*Rainfall Data.* The rainfall record for this period at the places where the wheats were grown is given in Table VII. This record indicates a dry period before the rains started on May 31. For most stations the interval between the date of heading and date of harvest was over one month. In most cases no rain occurred immediately after harvesting and hence the effects observed were due to the rain which fell while the wheat was standing in the fields. The rains which fell at Hutchinson on the two days after harvesting had no effect, since the wheat was combined.

*Results.* The data on test weight, flour yield, and ash, given in Table VIII, were obtained from a project underway to study the

TABLE VIII  
TEST WEIGHT, FLOUR YIELD, AND ASH VALUES FOR WHEAT SAMPLES  
GROWN AT VARIOUS LOCATIONS IN KANSAS

Place grown	Rain	Test weight			Flour yield and flour ash					
		Black-hull	Chiefkan	Tenmarq	Blackhull		Chiefkan		Tenmarq	
					Yield	Ash	Yield	Ash	Yield	Ash
	<i>inches</i>	<i>lb</i>	<i>lb</i>	<i>lb</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>
Manhattan	14.11	55.2	57.0	54.2	71.4	0.39	73.4	0.44	74.2	0.43
Thayer	13.32	56.5	—	57.9	68.8	0.43	—	—	72.8	0.42
Hays	11.45	57.5	60.6	56.2	69.0	0.42	73.0	0.43	71.8	0.42
Hutchinson	10.92	59.6	60.5	58.4	72.0	0.40	71.3	0.42	71.8	0.42
Columbus	7.84	54.5	—	55.2	67.3	0.42	—	—	73.5	0.43
Kingman	6.74	57.9	58.5	55.8	70.5	0.43	72.0	0.43	73.0	0.42
Tribune	6.27	—	61.0	58.9	—	—	72.5	0.43	72.0	0.43
Meade	5.91	59.2	59.5	56.9	70.8	0.44	71.6	0.46	72.0	0.44
Colby	4.19	58.3	61.6	57.9	71.0	0.43	73.3	0.42	73.0	0.42
Garden City	3.50	—	59.8	55.7	—	—	69.0	0.51	71.4	0.46
Dodge City	2.98	57.4	60.3	52.5	71.0	0.43	72.8	0.47	68.5	0.46

influence of environment on the quality of wheat varieties. One weakness in the data of Table VIII is that it is not known what figures would have been obtained in the absence of these rains. Comparisons can therefore be made only with what is generally obtained on unweathered wheat. On this basis the flour yields given in Table VIII are as high from wheats exposed to these rains as would be obtained from wheats not so exposed. The ash figures are also about what would be expected from wheat ripened under drier conditions.

The figures for internal texture given in Table IX show that the Blackhull samples were the least vitreous or most mealy, with the Tenmarq samples intermediate. Thus, the samples grown in various parts of the state show substantially the same effects from the rains

TABLE IX  
INTERNAL TEXTURE VALUES FOR WHEAT SAMPLES GROWN AT VARIOUS LOCATIONS  
IN KANSAS

Place grown	Rain	Blackhull			Chiefkan			Tenmarq		
		Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy	Vit.	Semi vit.	Mealy
	<i>inches</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>
Manhattan	14.11	11	44	45	71	23	6	6	53	41
Thayer	13.32	53	31	16	—	—	—	74	15	11
Hays	11.45	53	38	9	96	4	0	69	25	6
Hutchinson	10.92	49	41	10	99	1	0	90	8	2
Columbus	7.84	47	25	28	—	—	—	64	14	22
Kingman	6.74	22	57	21	70	25	5	52	31	17
Tribune	6.27	—	—	—	96	0	4	93	7	0
Meade	5.91	58	31	11	90	7	3	72	19	9
Colby	4.19	36	50	14	97	2	1	88	10	2
Garden City	3.50	—	—	—	89	10	1	64	33	3
Dodge City	2.98	69	26	5	90	7	3	72	19	9

as those from Manhattan. There seems to be no consistent relationship between the size of the rains and the effects, as shown by the results from places of largest, medium, and smallest rainfall. This is indicated by the results from the three groups in Tables VIII and IX. That is, frequent small rains will decrease the test weight and change the internal texture as well as the larger rains.

The protein and loaf volumes given in Table X, also obtained from the project on influence of environment, show that Chiefkan was the

TABLE X  
PROTEIN AND LOAF VOLUME OF FLOURS MILLED FROM WHEATS GROWN AT VARIOUS  
LOCATIONS IN KANSAS

Place grown	Blackhull		Chiefkan		Tenmarq	
	Protein <sup>1</sup>	Loaf vol.	Protein <sup>1</sup>	Loaf vol.	Protein <sup>1</sup>	Loaf vol.
	<i>%</i>	<i>cc</i>	<i>%</i>	<i>cc</i>	<i>%</i>	<i>cc</i>
Manhattan	12.4	843	13.6	783	12.8	943
Thayer	12.9	847	—	—	11.4	770
Hays	16.4	900	16.2	825	16.0	1142
Hutchinson	13.0	841	12.7	740	12.4	797
Columbus	10.5	702	12.8	—	9.8	683
Kingman	13.1	837	14.8	743	13.2	915
Tribune	—	—	16.1	733	14.7	958
Meade	15.7	927	—	888	14.8	1000
Colby	14.6	912	13.3	847	13.3	900
Garden City	—	—	18.3	915	18.0	1315
Dodge City	15.1	958	15.1	898	15.6	1207

<sup>1</sup> Protein content ( $N \times 5.7$ ) is expressed on the "as is" moisture basis.

poorest in baking value although it was the best from the grain-grading standpoint, as shown by the test weight and texture values given in Tables VIII and IX. Tenmarq gave the largest loaf volumes except when it was lower in protein. All the loaves from Blackhull were larger than the comparable samples from Chiefkan.

The figures for protein in Table X show also that high protein wheat is not inconsistent with a comparatively large rainfall during heading.

### Summary and Discussion

The data which have been presented on the effects of frequent rains accompanied by storms during the heading and ripening period indicate the same general effects as were obtained from the smaller rains reported previously (Swanson, 1943a). The larger rains caused greater mechanical losses in the field, but these were not reflected in the quality. The results of soaking wheat in the heads and in the grain indicate that the glumes afford some protection against the entrance of water into the kernels by absorption, such as takes place in soaking grain. The entrance of water into the interior of the kernels seems to be by molecular diffusion as was discussed in the previous report (Swanson, 1943a). Thus a small rain at night, or one followed by cool, cloudy weather may have as much effect as a larger rain followed by sunshine.

The data presented in this and the previous papers (Swanson, 1943, 1943b) show that lowering of test weights due to rains on wheat in the field, does not decrease the flour yield, increase the ash, nor lower the baking value. Sprouting was not observed in these samples. The size of the rains, except for mechanical losses, is of less consequence than the duration of the period of wetness which allows the molecular diffusion of water into the kernels. The causes of the lowering of test weight, decrease in the vitreous condition, and increase in the mealy texture are the loosening of the bran layer and the disturbing of the structure and arrangements of the material in the interior of the kernels as has been explained in previous papers (Swanson, 1941, 1943a).

These studies indicate that too much emphasis is placed on test weight in grading weathered wheat. This is of greater significance now because combining has become the prevailing method of harvesting wheat. The present grain grades were established when the grain binder was the common harvesting implement. Cutting could then be started when the grain had as much as 30 to 35% moisture.

Wetting and drying the grain before it has become hard has comparatively little effect on milling qualities. When the combine is used, the moisture should be about 13% and preferably less. Frequent rains on such wheat will lower the test weight with consequent de-

pression in grade. That the farmer who has weathered wheat is unduly penalized because major emphasis is placed on test weight in grading is clearly shown by the results of these investigations. These studies have indicated that the lowered wheat grades, due to rain, are not correspondingly reflected in decreased flour yields nor in baking values.

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## FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH. VI. EFFECT OF CYSTEINE AND SOME OTHER SUBSTANCES ON MIXOGRAM PATTERNS<sup>1</sup>

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In a previous publication (Swanson, 1940, 1940a), it was shown that the pattern of curves or mixograms (Swanson and Johnson, 1943) was markedly influenced by dough rest periods as well as by the presence of cysteine monohydrochloride, the action of cysteine<sup>2</sup> being very vigorous. The main effect of cysteine was a shortening of the developing and the weakening periods as shown by steeper ascending and descending slopes of the mixogram patterns. Swanson and Dines (1939) found that the addition of cysteine in the preparation of the doughball for

<sup>1</sup> Contribution No. 100, Department of Milling Industry, and No. 281, Department of Chemistry.

<sup>2</sup> For brevity, cysteine monochloride will be referred to as cysteine throughout this paper.

the wheat-meal time test notably shortened the time required for disintegration to start and this was in direct proportion to the amount of cysteine added. In this respect the action of cysteine was similar to that of proteases (Swanson, 1939).

The effects of glutathione and cysteine on farinograms and baking were included in the studies by Sullivan, Howe, and Schmalz (1936). Swanson and Andrews (1942, 1943) have shown that the presence of certain surface-active or wetting agents markedly lengthens the developing and weakening periods. The wetting agents acted mostly, if not altogether, on the gluten materials as indicated by the characteristics of patterns obtained from mixtures of finely ground dry gluten and wheat starch.

The three main objects of the present investigation were to compare the influence on mixogram patterns of (1) cysteine when used alone and in several combinations with the wetting agent, Aerosol OT (sodium dioctylsulfosuccinate), and with sodium chloride; (2) cysteine and hydrogen sulfide, ethyl mercaptan, and isopropyl mercaptan; and (3) cysteine and cystine. The effects of these agents were judged by the variations they produced in the mixogram patterns.

### Materials and Methods

Tenmarq flour of 13.3% protein was used for all the mixograms. This flour had a fairly long period of mixing, and hence was suitable for use with substances which shorten this time. For each mixogram, 35 g of flour and 22 ml of water or solution of the various substances under investigation were employed. In placing the substances in the mixing bowl the most active ingredients were added last so that their time of contact with the flour would be nearly the same as the mixing period. The amounts of the various agents are given in the legends to the figures presenting the mixograms.

Comparative baking tests were also made with Tenmarq and Chiefkan flour to determine the effects of Aerosol OT and cysteine on mixing time and loaf volume.

### Results

#### Effects on Mixogram Patterns

*Cysteine in Combination with Aerosol OT.* The opposite effects of cysteine and Aerosol OT are shown in the upper two lines of mixograms in Figure 1. Increasing amounts of cysteine shortened the time to reach minimum mobility from 3.5 minutes with water alone to 1.5 minutes with 10 mg of cysteine. The increasing amounts of Aerosol OT lengthened the time to about 7 minutes. Since these two substances influence the patterns in opposite directions, it would be ex-

pected that they would overcome each other's effects. The mixograms obtained with these two substances in conjunction (lines 3, 4, and 5) show the various patterns possible. As cysteine was increased, more and more Aerosol OT was required to overcome the effects of the cysteine, and when 9 mg were used, even the larger amounts of Aerosol OT did not suffice.

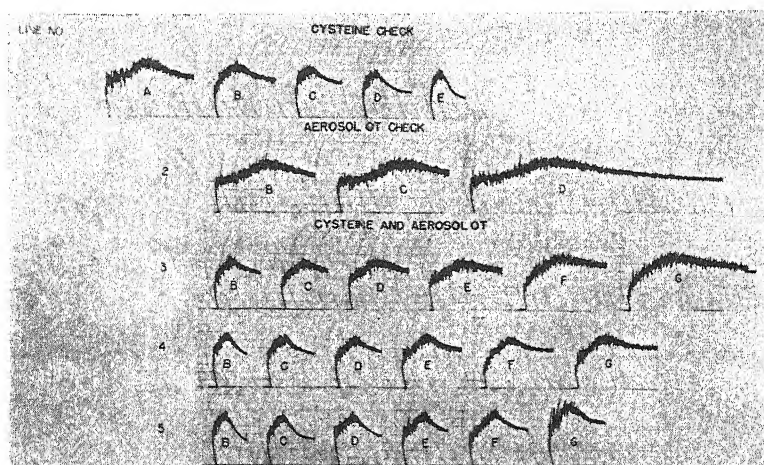


Fig. 1. Effect of cysteine, Aerosol OT, and combinations thereof on the mixogram pattern.

Line	Chemical added	A	B	C	D	E	F	G
		mg	mg	mg	mg	mg	mg	mg
1	Cysteine	0	2	4	6	10		
2	Aerosol OT		20	40	60			
3 <sup>1</sup>	Cysteine+Aerosol OT		3+0	3+20	3+40	3+60	3+80	3+100
4 <sup>1</sup>			6+0	6+20	6+40	6+60	6+80	6+100
5 <sup>1</sup>			9+0	9+20	9+40	9+60	9+80	9+100

<sup>1</sup> 1st figure in each column represents cysteine, 2nd figure represents Aerosol OT.

*Cysteine Alone and in Combination with Sodium Chloride.* The presence of various salts in the wash water for gluten was shown by Dill and Alsberg (1924) to influence notably the yield of gluten. Sharp and Gortner (1924) found that as dough fermentation by yeast progressed, it became more and more difficult to wash gluten from dough with distilled water, but with 1% sodium chloride solution as much gluten was obtained after 8 hours fermentation as from unfermented dough.

The stiffening effect of sodium chloride on dough is shown in the upper line of mixograms in Figure 2; the more salt that is used, the

greater the effect. When sodium chloride was combined with cysteine (lines 2, 3, and 4) the stiffening effects were still apparent, but unlike Aerosol OT (Fig. 1) there were no increases in the time required to reach minimum mobility. The patterns in Figures 1 and 2 show that although the effects of both sodium chloride and Aerosol OT persist in the presence of cysteine, their actions are different. This stiffening effect is apparently due to a change in the character of the water caused

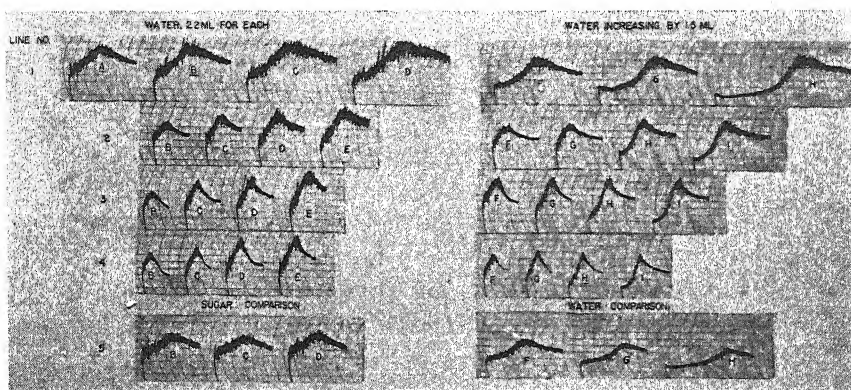


Fig. 2. Effect of cysteine in combination with sodium chloride on the mixogram pattern. Mixograms were made at constant absorption and also at augmented absorptions in proportion to the quantity of sodium chloride added. For comparative purposes, mixograms are shown for flour-water and flour-water-sucrose mixtures.

Line	Volume of solution constant					Water increment 1.5 ml per 0.35 g NaCl			
	A	B	C	D	E	F	G	H	I
1 <sup>1</sup>	0+0	0+0.35	0+0.70	0+1.05		0+0.35	0+0.70	0+1.05	
2 <sup>1</sup>		3+0.0	3+0.35	3+0.70	3+1.05	3+0.0	3+0.35	3+0.70	3+1.05
3 <sup>1</sup>		6+0.0	6+0.35	6+0.70	6+1.05	6+0.0	6+0.35	6+0.70	6+1.05
4 <sup>1</sup>		9+0.0	9+0.35	9+0.70	9+1.05	9+0.0	9+0.35	9+0.70	9+1.05
5 <sup>2</sup>	0.70	1.40	2.1				23.5	25	26.5

<sup>1</sup> In lines 1 to 4 inclusive, first figure in each column represents mg cysteine, second figure represents g NaCl.

<sup>2</sup> Figures in Columns A, B, C represent grams sucrose. Figures in columns G, H, I represent ml water alone.

by a possible appropriation of the water by the ions from the sodium chloride. This is indicated in the patterns to the right. The addition of 1.5 ml of water for each 0.35 g of sodium chloride does not reduce the pattern heights, but with the slacker dough more and more time is needed to reach the peak. This stiffening or increase in plasticity appears to result from the binding of the water molecules to the ions from sodium chloride. With sodium chloride the plasticity is much decreased as can be seen by the decreased heights of the last three



patterns on line 5. In contrast to sodium chloride, sucrose has apparently little effect on plasticity.

*Sulphydryl Compounds.* The powerful action of cysteine upon dough would seem to indicate that the cysteine molecule has a structural group peculiarly active in effecting changes in the protein. Of the various groups in the cysteine molecule the -SH group would seem to be of the most importance in causing these changes. Three other substances, hydrogen sulfide, ethyl mercaptan, and isopropyl mercaptan, which contain such groups, were used in this study.

The effects of the presence of -SH groups on flour and dough properties have been studied by Balls and Hale (1936, 1936a) and by Sullivan, Howe, and Schmalz (1936). Ziegler (1940, 1940a, 1940b), as well as others, has included glutathione in his studies of the changes which take place in dough.

The mixograms in line 1 of Figure 3 indicate that cysteine acts principally on the gluten proteins. Gluten was prepared from the Tenmarq flour used for the previous mixograms and blended with wheat starch, employing the method of Swanson and Andrews (1943). The protein content of this gluten was 77.5% on the air-dry basis, which indicates that considerable amounts of nongluten substances were present. Their influence was probably not important, as preliminary trials indicated that 8 g of air-dry gluten, finely ground, 27 g commercial wheat starch,<sup>3</sup> and 25 ml of water would give mixograms similar to those obtained from flour alone. This is shown by A in line 1. The effects of increasing amounts of cysteine are shown in the other mixograms in this line.

Cysteine is related to cystine by the union of two cysteine molecules through a disulfide linkage. Increasing amounts of a 5% solution of cystine in dilute HCl (line 2) do not show effects comparable to those of cysteine. The mixograms in line 3 were made with increasing amounts of 1.0*N* HCl and the patterns obtained show a strong similarity to those in line 2. This indicates that the small effect from cystine was due to the HCl used as a solvent.

The mixogram patterns obtained with saturated aqueous solutions of hydrogen sulfide, ethyl mercaptan, and isopropyl mercaptan shown in Figure 3 strongly resemble those obtained with cysteine (Figs. 1 and 2). These results indicate that the -SH group, which is common to these different substances, is the main causative agent in influencing the mixogram patterns. A few trials made with  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  gave similar patterns.

<sup>3</sup> This starch was furnished by the Huron Milling Co., Huron, Michigan.

## Effects on Baking

Only a few trials have been made on the effects of a wetting agent and cysteine on baking results. The loaves in Figure 4 which were baked by a rich formula containing dry milk and shortening show some possibilities.<sup>4</sup> The addition of 200 mg of Aerosol OT per loaf (100 g

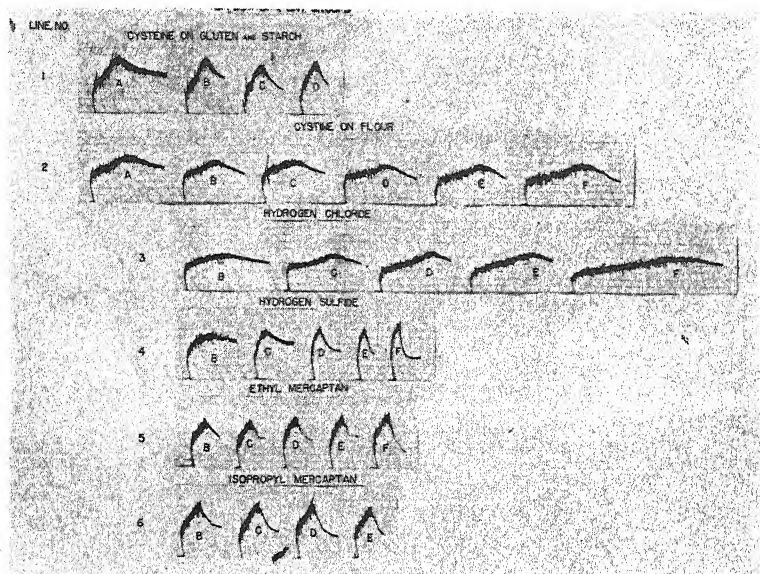


Fig. 3. Effect of hydrogen chloride and various sulfur-containing compounds on the mixogram pattern.

Line	Chemical added	A	B	C	D	E	F
1	Cysteine mg <sup>1</sup>	0	4	8	12		
2	Cystine ml 5% sol.	0	1	2	3	4	6
3	Hydrogen chloride ml 1.0N sol.		1	2	3	4	6
4	Hydrogen sulfide ml sat. sol.		1	3	6	10	15
5	Ethyl mercaptan ml sat. sol.		1	2	3	4	5
6	Isopropyl mercaptan ml sat. sol.		3	5	7	10	

<sup>1</sup> The cysteine was used on mixtures of dried, finely ground gluten and starch.

flour) approximately doubled the mixing time for doughs prepared from both Tenmarq and Chiefkan flours, whereas the addition of 8 mg of cysteine per 100 g of Tenmarq flour reduced the mixing time to 2.0 minutes, as compared with 3.4 minutes for the control.

The loaves containing Aerosol OT and cysteine are equal to the checks. Thus, decreasing the mixing time of Tenmarq with cysteine

<sup>4</sup> The authors are indebted to Mr. John A. Johnson, Assistant Baking Technologist, for making the baking tests.

and increasing that of Chiefkan with Aerosol OT resulted in as good volume and texture as when these substances were not present. This indicates the possibilities of altering the mixing time and still obtaining good bread. Use of cysteine and Aerosol OT in baking is being investigated further.



Fig. 4. Typical loaves, showing the effect of cysteine and Aerosol OT on baking properties of flour.

Loaf No.	Treatment	Mixing time	Loaf volume
		<i>min</i>	<i>cc</i>
1	Check, Tenmarq (13.3% protein)	3.4	930
2	200 mg Aerosol OT	6.4	928
3	Check, Chiefkan (13.0% protein)	1.7	728
4	200 mg Aerosol OT	3.5	778
5	8 mg cysteine	2.0	933

### Discussion

In evaluating the magnitude of the opposite influence of Aerosol OT and cysteine on mixogram patterns, it becomes apparent that the effects are proportional to the molecular concentration of these substances. The concentrations were adjusted to procure pen swings of comparable magnitude and similar shape. The amounts thus used, as indicated in the figure legends, seem to bear little relationship until calculated on the molecular basis. As the molecular weight of Aerosol OT is about 3.7 times that of cysteine, it would require approximately 3.7 times the

weight of Aerosol OT to produce the equivalent effect of cysteine if each were of equal potency in their action upon dough.

Sodium chloride increases the dough stiffness, as shown by the increased mixogram heights, but the stiffening effects were naturally much less when the water was increased by 1.5 ml for each 0.35 g of sodium chloride used. Sodium chloride also tends to minimize the effects of cysteine. That the action of cysteine results from the -SH group is indicated by the similar effects obtained with hydrogen sulfide, ethyl mercaptan, and isopropyl mercaptan, all of which contain -SH groups.

Certain selected mercaptan patterns could be exactly superimposed upon certain cysteine patterns. The same was true for hydrogen sulfide. Since no alteration in curve pattern was obtained with the addition of cystine, it seems that the -SH group, rather than the -NH<sub>2</sub> or -COOH group, is the one which is active in altering dough characteristics.

The main effects of cysteine and of the substances which had similar results on the mixogram patterns are shown in steeper slopes; the heights are not greatly decreased. This indicates that the water makes contact with the gluten more quickly. But as soon as the dough has attained its maximum stiffness, weakening or slackening starts and proceeds at a much faster rate than for a corresponding flour-water dough. The behavior on the downslope suggests that the amounts of free water in the dough begin to increase as soon as the peak is passed, and this process gradually continues, as shown by the progressive slackening of the dough. The addition of cysteine to Tenmarq produced a mixogram similar to that for Chiefkan flour-water dough.

The counteracting effects of sodium chloride when used with cysteine may be due to an association of the polar water molecules with the ions from sodium chloride, producing ion hydration (Gortner, 1938). The more water molecules which are thus associated, the fewer there are which are free in the water films to influence dough mobility. The degree of mobility of the dough results from the varying freedom of the water molecules in the layers of water which cover the gluten strands or the starch granules. The thicker these layers, as related to the water absorption, the greater the mobility of the dough. Workers in this laboratory have found that increases in absorption will decrease the heights of mixograms but increase the time required to reach minimum mobility. This increased mobility has also been observed when sodium chloride is used and the amount of water is increased.

The stiffening action of sodium chloride upon dough seems to indicate a salting-out effect upon the protein. As the proteins become

salted out, they tend to lose their own water of hydration to the stronger hydration forces around the ions from the sodium chloride (Debye, 1927). Thus the protein strands seem to behave as though separated by water layers which have less freedom of molecular movement and the protein itself seems to become less highly hydrated and to stiffen.

### Summary

Mixogram patterns can be markedly changed by the use of certain substances. Cysteine decreases the time to reach minimum mobility, while Aerosol OT (sodium dioctylsulfosuccinate) produces opposite results. Thus, by a suitable choice of the amounts of these reagents, the time factor in the mixogram pattern may be made longer or shorter.

Sodium chloride has a stiffening effect on the dough, as indicated by increased heights of the mixograms, and this action persists in the presence of cysteine. This stiffening effect was minimized by increasing the water by 1.5 ml for each 0.35 g of sodium chloride added.

Other substances containing -SH groups, such as hydrogen sulfide, ethyl mercaptan, and isopropyl mercaptan, influenced the mixogram pattern in a manner similar to cysteine. This indicates that the -SH group in cysteine is the main cause of its action on dough.

The few results obtained in baking indicated that the presence of cysteine or Aerosol OT, in the amounts used, had no deleterious effects on loaf volume or texture.

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## EFFECT OF HYDROCYANIC ACID ON THE BAKING QUALITY OF FLOUR

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(Received for publication November 4, 1943)

The extensive use of hydrocyanic acid as a fumigant for flour makes the question of its retention by that material one of great importance. Marchadier *et al* (1921) reported finding 82 ppm of hydrocyanic acid in flour and stated that food prepared from this flour tasted of cherry laur<sup>1</sup>.

Griffin *et al* (1923), using sodium cyanide and the pot method of generation in dosages of from 1 to 6 oz per 100 cu ft of space, found up to 200 ppm of hydrocyanic acid in flour immediately after fumigation. However, after 4 days' storage in a large, well-ventilated room at 70°F practically all the hydrocyanic acid had left the flour.

Moucka (1936) fumigated wheat and rye flour for 48 hours with 1% of hydrocyanic acid gas by volume. Immediately after treatment the wheat flour showed 3-4 ppm, the rye flour 7-9 ppm. After 24 hours' aeration both were completely free from hydrocyanic acid.

Dean and Swanson (1911) published on the effect of common mill fumigants on the baking qualities of wheat flour. They fumigated three grades of hard winter wheat flour and four grades of soft winter wheat flour with hydrocyanic acid generated from potassium cyanide at the rate of 1 lb per 1000 cu ft of space for 12 hours at 90°F. Baking

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tests were made immediately after fumigation, repeated 2 or 3 days later, and again repeated after intervals of 30 and 60 days. They conclude, "An examination of the tables and plates will show that the effects of fumigation are so small as to be negligible. It is only in the careful measurements employed in the test that any difference between the fumigated and unfumigated flour is apparent at all. The only notable difference appears in the maximum volume of the dough in the test made immediately after the fumigation, but not after 30 days. The finished loaf shows no deleterious effect from fumigation in any of the tests."

Very little work has been reported in the literature on the effect of hydrocyanic acid on the baking quality of flour. The results reported at this time are limited but it appeared that they would be of interest to handlers of food products. Increased emphasis is now placed upon freedom of such products from insect life.

### Experimental

The first experiments were conducted with a prepared ginger-cake flour. This flour was packed in 12-oz cartons and wrapped in cellophane. The packaged flour was fumigated in a vacuum vault of 2550-cu ft capacity for 18 hours with a total dosage of 5 lb of liquid hydrocyanic acid and a vacuum of 26 inches.

Immediately after the vault was unloaded a package was emptied into a glass jar and sealed. As soon as possible it was analyzed for the hydrocyanic acid retained. The hydrocyanic acid was determined by distilling it from a 20-g sample of flour to which 1 g of tartaric acid had been added. The HCN in the distillate was titrated with silver nitrate by the well-known Liebig method. Other samples were taken at 24-hour intervals for analysis from packages stored in the warehouse. Figure 1 presents the data for these analyses. All results are based on two closely agreeing analyses. The high initial concentration and the rapid loss of hydrocyanic acid are very interesting. The entire package at the highest concentration would contain 0.05 g of hydrocyanic acid. According to the figure given by Gettler and Baine (1938) as to the minimum lethal dosage (MLD) of hydrocyanic acid this would be about one-half the MLD for an adult.

Samples of the prepared ginger-cake flour were taken from the carton sealed immediately after the fumigation and baked. The cake was analyzed for hydrocyanic acid, but none was found. The rest of the cake was eaten by the laboratory staff with no ill effects.

Reports have reached the writers lately of difficulty experienced by bakers using recently fumigated flour. In order to determine what interval of time must elapse between fumigation and the complete loss

of hydrocyanic acid the following work was undertaken: A 48-lb bag of patent flour was fumigated for 24 hours in a vault at normal atmospheric pressure with a dosage of 2.25 oz of liquid hydrocyanic acid per 1000 cu ft of space. This low dosage was adopted so that the single bag used would absorb approximately the same quantity as would one of a warehouseful during a normal fumigation. Immediately on its

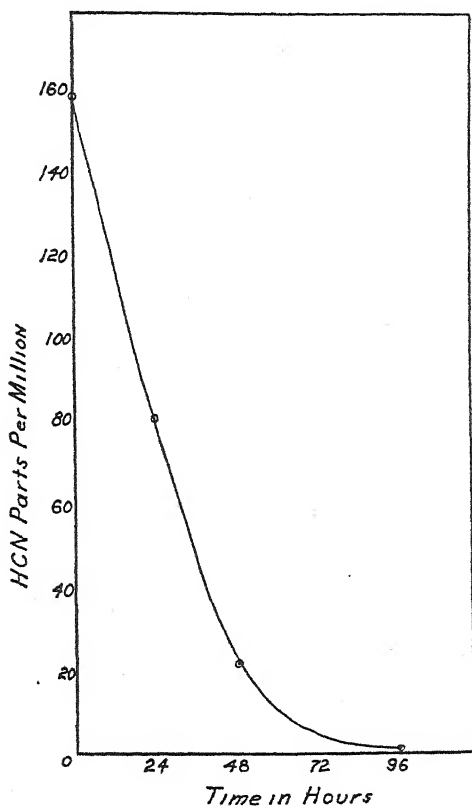


Fig. 1. Loss of hydrogen cyanide from fumigated prepared cake flour.

removal from the vault the bag of flour was sampled, and other samples were taken at 24-hour intervals thereafter. The results are represented in Figure 2. The samples were baked using 3% shortening superimposed upon the lean formula and procedure described in detail by Johnson, Swanson, and Bayfield (1943). Results are presented in Table I.

The volume of the loaf may be seen to have been definitely depressed in the sample taken immediately after fumigation, amounting



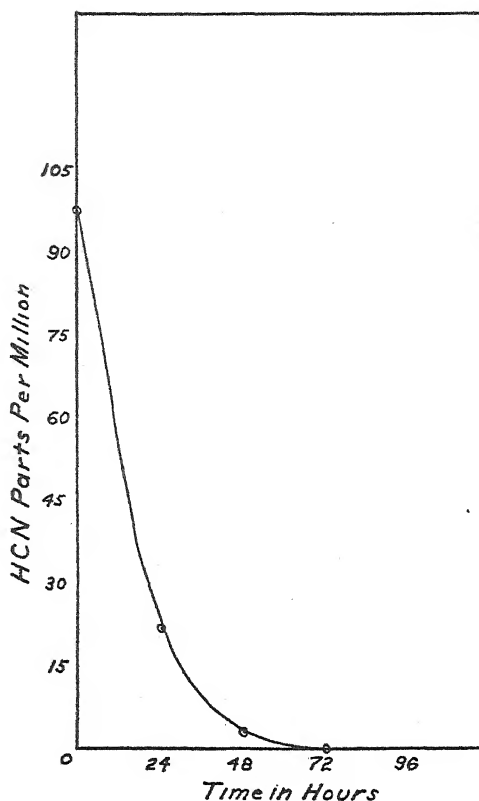


Fig. 2. Loss of hydrogen cyanide from fumigated bread flour.

to about 11% of the check. After 24 hours the volume was still somewhat depressed. The differences shown by the 48- and 72-hour samples are not significant. No odor of hydrocyanic acid could be

TABLE I  
BAKING RESULTS AFTER FUMIGATION WITH HYDROGEN CYANIDE<sup>1</sup>

Aeration	Mixing time	Loaf volume	Crumb color <sup>2</sup>	Crumb grain and texture <sup>3</sup>
<i>hours</i>	<i>min</i>	<i>cc</i>		
0	2.0	888	82 cy	80-o
24	2.5	945	85 cy	83-o
48	2.7	990	87 cw	87-o
72	3.3	1015	87 cw	85-o
Check	3.5	1000	87 cw	85-o

<sup>1</sup> Flour protein 12% (15% moisture basis), moisture 10.9%, absorption 68%. Results are averages of three fumigations.

<sup>2</sup> Cy = creamy yellow; cw = creamy white.

<sup>3</sup> o = open grain.

detected in the baked loaves. Since this odor test is very sensitive, no analysis of the baked loaves was deemed necessary.

The difference in volume of the baked loaves, as shown in Figure 3, is readily observable. The consistency of the dough made from the

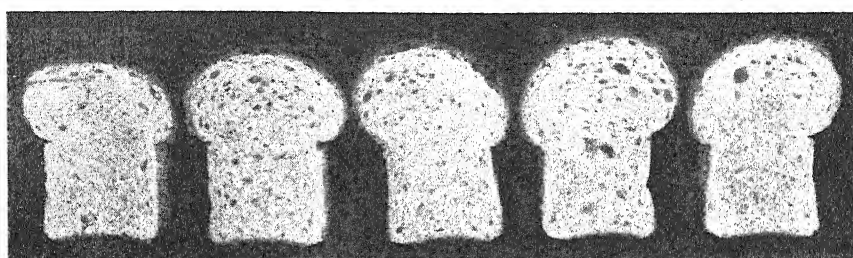


Fig. 3. Loaves of bread baked from fumigated flour at various intervals after fumigation with hydrogen cyanide.

different flours is shown in the "mixograms" presented in Figure 4. The nature of these recordings is completely described by Swanson and Johnson (1943). Briefly, they are graphs or curves made by a recording dough mixer known as a mixograph. Each graph constitutes

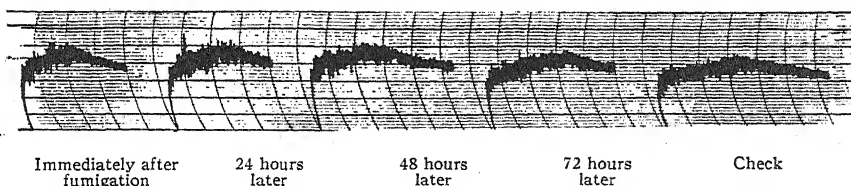


Fig. 4. Mixograms of fumigated flour, at various intervals after fumigation with hydrogen cyanide.

a record of the behavior of a dough during mixing and development by mechanical action. This record includes the rate of dough development, the maximum stiffness or resistance at complete mixing or minimum mobility, the tolerance or sensitiveness to mixing, and the rate of break-down or increase in mobility. Each vertical line on the mixograms represents an interval of approximately 1 minute. It may be seen from these curves that the effect of the hydrocyanic acid is directly on the flour, since they were obtained on the flour and water alone. These curves show a reduction in mixing time and a tendency toward increased slackness in the dough due to absorbed fumigant.

This work indicates that a baker about to have his warehouse fumigated should hold out enough unfumigated flour to last him for 3 days' baking to avoid trouble from the absorbed fumigant. It is likely that the HCN residue would not be lost so rapidly from a stack of bagged

flour as from a single sack as used in these tests. Therefore, fumigated flour should be thoroughly aerated before it is used in baking.

### Summary

Hydrocyanic acid when used as a flour fumigant produces an appreciable and detrimental influence upon the bread baking quality. This effect is not apparent after thorough aeration of the flour.

Ginger cake from freshly fumigated flour was free of fumigant.

### Acknowledgment

The authors are indebted to John A. Johnson for making the baking tests.

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## NICOTINIC ACID IN PRODUCTS OF COMMERCIAL RICE MILLING AND IN RICE VARIETIES<sup>1</sup>

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In previous communications (Kik, 1943; Kik and Van Landingham, 1943) studies were reported on thiamine and riboflavin in products of commercial rice milling and in rice varieties. This investigation deals with the nicotinic acid content of these products. In addition, samples of parboiled and undermilled rice have been tested.

The nicotinic acid content of wild rice has been reported by Nelson and Palmer (1942), and Williams, Knox, and Fieger (1943) recently

<sup>1</sup> Research paper No. 781, Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Experiment Station. Aided by a grant from the Williams-Waterman Fund of the Research Corporation.

<sup>2</sup> Resigned.

published a study of the vitamin B-complex factors in rice and its milled products.

The essential steps in milling, description of the main products and by-products, and the methods employed in obtaining representative samples were described in our previous papers. Nicotinic acid was determined by the colorimetric method of Melnick (1942); the analyses were made in duplicate, employing 1.0 g samples of the main products and 250 mg of the by-products. As a test of the suitability of the colorimetric method, the samples of whole brown rice employed in this study were submitted to another laboratory for microbiological

TABLE I  
NICOTINIC ACID IN PRODUCTS OF COMMERCIAL RICE MILLING<sup>1</sup>

Products	Variety <sup>2</sup> and mill lot							
	Supreme Blue Rose		Early Prolific		Fortuna	Lady Wright	Improved Blue Rose <sup>4</sup>	Mean
	551	606	569	663	635	768	778	
NICOTINIC ACID CONTENT (DRY MATTER BASIS)								
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Paddy or rough rice	49.8	49.8	48.5	47.7	46.4	55.4	47.3	49.2
From milling or bleaching process:								
Whole brown rice <sup>5</sup>	57.4	56.4	51.2	52.8	49.2	64.7	54.3	55.1
First break huller rice	25.0	25.2	22.8	21.2	22.3	24.6	24.9	23.7
Second break huller rice	24.5	23.3	21.6	20.9	21.4	20.2	24.8	22.4
Pearling cone rice	24.0	23.0	21.0	20.7	— <sup>3</sup>	— <sup>3</sup>	24.4	22.6
Brush rice	22.0	21.0	20.7	20.3	17.4	20.0	22.0	20.4
Finished, clean, polished rice:								
Head rice	18.9	17.5	20.0	19.7	15.6	19.5	18.0	18.4
Second head rice	17.2	16.5	19.5	19.1	15.5	18.9	15.6	17.7
Screenings	24.4	23.2	24.3	25.1	22.8	24.1	22.6	23.8
Brewers' rice	39.5	37.8	34.4	35.0	34.9	37.1	33.9	36.1
Rice by-products:								
Hulls	16.9	17.3	17.0	17.9	25.1	22.1	14.0	18.6
First break bran	320.0	338.0	340.8	358.2	315.1	303.2	349.0	332.0
Second break bran	306.0	316.0	259.5	265.1	283.1	262.8	311.7	286.3
Pearling cone polish	408.0	412.0	359.0	347.5	— <sup>3</sup>	— <sup>3</sup>	312.5	367.8
Brush polish	384.4	368.0	269.4	232.3	206.6	296.0	275.9	290.4

<sup>1</sup> Obtained from one mill through the courtesy of the Walton Mill, Inc., Stuttgart, Arkansas.

<sup>2</sup> All varieties are from fields which were not fertilized and were irrigated by well water.

<sup>3</sup> Pearling cones are not used in long grain varieties.

<sup>4</sup> Grown in Louisiana.

<sup>5</sup> The following values were obtained by John S. Andrews, Research Department, General Mills Inc., for the same samples of whole brown rice tested by the microbiological method: 57.1, 55.1, 53.3, 47.3, 53.5, 58.4, and 50.0. Average 53.5 µg/g. These values are in good agreement with those obtained by the colorimetric method.

assay.<sup>3</sup> For these assays the method described by Andrews, Boyd, and Gortner (1942) was used.

### Results

Table I shows that on the dry basis rough rice contained an average of 49.2  $\mu\text{g/g}$  of nicotinic acid, while brown rice contained 55.1  $\mu\text{g}$  or slightly more than rough rice. An average of 53.5  $\mu\text{g}$  was obtained for the same samples of whole brown rice, using the microbiological method, thus confirming the validity of the colorimetric method for the determination of nicotinic acid in these samples.

The decrease in mean percentage of nicotinic acid in converting brown rice to head rice amounted to 66.4%. These decreases for individual varieties ranged from 60.9% for Early Prolific to 70.0% for Lady Wright.

TABLE II  
NICOTINIC ACID CONTENT OF MILLED PARBOILED AND MILLED NONPARBOILED RICE, MILLED AND UNDERMILLED RICE

Variety	Nicotinic acid content (dry matter basis) for stated treatment			
	Milled, parboiled	Milled, not parboiled	Undermilled	Milled
	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
Nira <sup>1</sup>	49.0	—	—	—
Nira <sup>1</sup>	—	20.6	—	—
Caloro <sup>2</sup>	45.2	—	—	—
Caloro <sup>2</sup>	—	18.5	—	—
Indian <sup>3</sup>	45.0	—	—	—
Lady Wright <sup>3</sup>	—	—	26.2	—
Lady Wright <sup>3</sup>	—	—	—	19.5
Supreme Blue Rose <sup>3</sup>	—	—	26.6	—
Supreme Blue Rose <sup>3</sup>	—	—	—	18.9

<sup>1</sup> Obtained through the courtesy of C. R. Adair, Associate Agronomist, U. S. Department of Agriculture, Bureau of Plant Industry, Rice Branch Experiment Station, Stuttgart, Arkansas.

<sup>2</sup> Obtained through the courtesy of the Rice Growers Association of California, Sacramento, California.

<sup>3</sup> Obtained through the courtesy of the Arkansas Rice Growers Cooperative Association, Stuttgart, Arkansas.

The results of assays of a few samples of parboiled and undermilled rice are shown in Table II, and indicate that the nicotinic acid content of milled parboiled rice is considerably higher than that of milled non-parboiled rice. A sample of milled parboiled rice of the Nira variety contained 49.0  $\mu\text{g/g}$  and a sample of milled parboiled California rice (Caloro variety) had 45.2  $\mu\text{g/g}$  compared with an average of 18.5  $\mu\text{g/g}$  for ordinary milled rices (Table I).

The nicotinic acid content of undermilled rice is higher than that of ordinary milled rice. An undermilled sample of Lady Wright con-

<sup>3</sup> Credit is due John S. Andrews, Research Department, General Mills, Inc., Minneapolis, Minnesota, for the Microbiological Assays of these samples.

tained 26.2  $\mu\text{g/g}$  compared with 19.5  $\mu\text{g/g}$  for a representative sample of milled rice.

Samples of 18 varieties of rough rice or paddy, obtained from the main rice-producing states, Arkansas, Louisiana, Texas, and California, were tested for their nicotinic acid content with the results shown in Table III.

TABLE III

NICOTINIC ACID CONTENT OF DIFFERENT VARIETIES OF PADDY OR ROUGH RICE GROWN IN ARKANSAS, LOUISIANA, TEXAS, AND CALIFORNIA (1941 HARVEST)

Variety	Nicotinic acid content (dry matter basis)			
	Arkansas <sup>1</sup>	Louisiana <sup>2</sup>	Texas <sup>3</sup>	California <sup>4</sup>
	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
Early Prolific	47.8	39.3	46.3	40.0
Caloro	46.0	49.6	52.6	—
Arkrose	55.7	48.6	—	—
Acadia	40.0	41.3	—	—
Prelude	41.5	43.2	—	—
Supreme Blue Rose	46.0	—	—	—
Improved Blue Rose	—	50.1	—	—
Blue Rose	—	—	46.5	—
Blue Rose	—	—	40.6	—
Blue Rose	—	—	44.2	—
Lady Wright	43.0	41.0	—	45.0
Nira	40.0	45.1	47.0	46.7
Arkansas-Fortuna	52.1	46.6	—	—
Zenith	53.3	56.8	52.0	48.6
Japan	—	—	52.4	—
Japan	—	—	46.8	—
Rexora	—	—	55.9	—
Rexora	—	—	54.7	—
Fortuna	—	—	59.0	—
Fortuna	—	—	51.1	—
Calady	—	—	—	40.7
Calady 40	—	—	—	43.4
Colusa	—	—	—	52.3
Average	46.5	46.1	49.9	45.2

<sup>1</sup> From Rice Branch Experiment Station, Stuttgart, Arkansas.

<sup>2</sup> From Rice Branch Experiment Station, Crowley, Louisiana.

<sup>3</sup> From Rice Grading Service, American Rice Growers' Cooperative Association, Beaumont, Texas.

<sup>4</sup> From Rice Experiment Station, Biggs, California.

The average nicotinic content of all varieties was 46.5  $\mu\text{g}$ ; the highest content (59.0) was found in a sample of Fortuna grown in California, and the lowest (39.3) was from a sample of Early Prolific grown in Louisiana. Small differences were found in the nicotinic acid content of varieties grown at the same location which indicates that varieties differ somewhat in their nicotinic acid content.

The apparent effect of locality on the nicotinic acid content is small. Early Prolific from four states showed similar nicotinic acid content; in Arkansas 47.8, Louisiana 39.3, Texas 46.3, and California 40.0.

Blue Rose grown in Texas in three different localities had a nicotinic acid content of 46.5, 40.6, and 44.2. Similar observations were made on the nicotinic acid content of the varieties Caloro, Lady Wright, Nira, and Zenith.

### Summary

The nicotinic acid content of products of commercial rice milling and of rice varieties has been determined. The average for paddy or rough rice and for whole brown rice was 49.2  $\mu\text{g/g}$  and 55.1  $\mu\text{g/g}$  respectively.

Of the finished, clean products, the end product, head rice (sold for human consumption), contained an average of 18.4  $\mu\text{g}$  and second head 17.7  $\mu\text{g/g}$  of dry matter. An average of 66.4% of nicotinic acid was removed during the milling process.

Screenings and brewers' rice contained 23.8 and 36.1  $\mu\text{g}$  nicotinic acid respectively.

Of the by-products, hulls contained 18.6  $\mu\text{g/g}$ , bran from 262.8 to 358.2  $\mu\text{g/g}$ , and rice polish 206.6 to 408.0  $\mu\text{g/g}$  of nicotinic acid. Three samples of milled parboiled rice (prepared in three different localities) contained 49.0, 45.2, and 45.0  $\mu\text{g}$  of nicotinic acid per gram of dry material. Two samples of undermilled rice contained 26.2 and 26.6  $\mu\text{g}$ , compared with 19.5  $\mu\text{g}$  and 18.9  $\mu\text{g/g}$  in the milled rice.

The average nicotinic acid content of all varieties (rough rice) was 46.5  $\mu\text{g/g}$ .

The nicotinic acid content of rice differed with variety and to a small extent with locality.

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## EXPERIMENTAL DESIGN FOR CEREAL CHEMISTS

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In the application to experiments of the principles of statistics, there is no clear line of demarcation between design and analysis. In order to think clearly about the design of a particular experiment it is necessary to have in mind the method of analysis that is to be used. Similarly, a study of different methods of analysis will suggest different designs. It is with this point in mind that certain suggestions are made in this paper that may be of value to experimentalists in cereal chemistry.

The intimate relation between experimental design and method of analysis is well illustrated by examples in which the end results are to be expressed in terms of correlation and regression. It is perhaps too frequently assumed that, in order to determine such relationships with a reasonable degree of accuracy, it is necessary to have a fairly large number of pairs of values in a homogeneous population. In order to illustrate this point, let us suppose that we are to determine the relation between the total nitrogen content of barley and the saccharifying activity of the malt extract expressed in degrees Lintner. In order to obtain a reliable measure of a correlation it is true that we should have a fairly large number of pairs of values, but it is not true that we must obtain these values from one variety or all from samples grown in one area. Methods of analysis are now available that enable us to group the results for a series of varieties and for samples of these varieties obtained in several areas. As a matter of fact, any determination of the correlation from one variety or from samples grown under a limited range of environmental conditions may be a pure waste of time. If different varieties give different correlations, it is perfectly clear that an experiment with one variety will not answer the question. Further, it seems quite possible that if the samples are all grown under conditions that give a uniform protein level, the relation determined may not be representative of the relation that actually exists in samples grown



under a wide range of climatic and soil conditions and giving a wide range of protein levels.

The same general principles should be kept in mind in almost any experiment. The correlation between protein and loaf volume might well be studied under different baking conditions, with different formulas, with different levels of the ingredients of the mix, and with different time elements entering into parts of the procedure. It is not desirable to include too many factors, as the experiment may become huge, unwieldy, and extremely complex; but it is always worth while to make a careful study of the various factors that might affect the results, and plan to vary at least those factors that are known from general experience to be important.

Experiments with more than one factor at different levels are usually referred to as factorial experiments. A great deal has been written on such experiments, especially in connection with field plot trials, and a considerable number of them may be found described in the literature of cereal chemistry. However, when two or more variables are involved in the study which otherwise is essentially similar to the factorial experiment, there is apparently a more limited knowledge with respect to appropriate methods of analysis. The procedure is generally known as the covariance analysis. One of the objects of this paper is to present this method by means of examples taken from cereal chemistry, and to illustrate where possible how a knowledge of the method may have a bearing on experimental design.

### Covariance Analysis

The covariance technique is well illustrated in a study conducted by Anderson *et al* (1939) on the correlation between total nitrogen and saccharifying activity of the malt extracts for 12 varieties of barley grown at 12 stations distributed across Canada. The 12 varieties were those most commonly grown in Canada, and there was at least one station in each of the important barley-growing districts.

The experiment provided 144 pairs of values of the two variables. If these are set up in the form of a scatter diagram, the scatter is considerable and does not indicate a very high value of the correlation coefficient. The actual value is 0.694. However, the material is very heterogeneous; and since part of the scatter may be due to this heterogeneity, the next step in the procedure is to determine the correlations for those components of the experiment that can be considered homogeneous. For example, we can determine a correlation coefficient for each variety, for the variety means, for the station means, or for all varieties combined after eliminating the effect of variety means and station means. The correlation coefficients determined for each va-

riety will be expected to show a good deal of variability, especially since they will be determined from only 12 pairs of values. It will be important, therefore, to make a test of the variation in these correlations. The procedures are illustrated below.

In the first place, we carry out the calculations necessary for setting up a preliminary analysis of covariance table, from which we can calculate some of the correlation and regression coefficients. These data are summarized in Table I, in which total nitrogen is represented by  $x$

TABLE I  
PRELIMINARY ANALYSIS OF COVARIANCE

	$\Sigma(x^2)$	$\Sigma(xy)$	$\Sigma(y^2)$	DF	Re- gres- sion	Residual	DF	$r_{xy}$	$b_{yx}$
Stations	18.1587	22.4636	30.1980	11	27.7891	2.4089	10	.959	1.2371
Varieties	0.8672	-0.09620	19.2276	11	0.0107	19.2169	10	-.024	-0.1109
Error	1.1296	1.4189	8.8040	121	1.7823	7.0217	120	.450	1.2561

and saccharifying activity by  $y$ ;  $x$  and  $y$  are measured in terms of deviations from their respective means, that is, in the table,  $\Sigma(x^2) = \Sigma(x - \bar{x})^2$ ,  $\Sigma xy = \Sigma(x - \bar{x})(y - \bar{y})$ , and  $\Sigma(y^2) = \Sigma(y - \bar{y})^2$ . The sums of squares of  $x$  and  $y$  are calculated as in the analysis of variance. The sums of products are calculated by an exactly analogous procedure. For example, the sum of products for stations is obtained by multiplying corresponding station totals for  $x$  and  $y$  and summing. This sum is divided by 12, the number of individual determinations entering into each total, and the correction term is found by multiplying the totals for  $x$  and  $y$  and dividing by 144.

The degrees of freedom in the 5th column represent the sums of squares of  $x$  and  $y$ . In the column headed "regression" we have that portion of the sum of squares of  $y$  that is accounted for by the linear regression. It is most easily calculated from  $[\Sigma(xy)]^2/\Sigma(x^2)$ , the values of  $\Sigma(xy)$  and  $\Sigma(x^2)$  being taken from the same line. From the sets of sums of squares and products we can calculate the correlation and regression coefficients. Thus—

$$r_{xy} = \frac{\Sigma(xy)}{\sqrt{\Sigma(x^2)\Sigma(y^2)}} \quad \text{and} \quad b_{yx} = \frac{\Sigma(xy)}{\Sigma(x^2)}$$

The "residual" is obtained by subtracting the sum of squares for regression from the sum of squares for  $y$  in the same line. Since each regression sum of squares represents one degree of freedom, the degrees of freedom given in the 8th column of the table are in all cases one less than the degrees of freedom for the original sums of squares of  $y$ . It is not necessary to calculate the 6th, 7th, and 8th columns in order

to determine the correlation and regression coefficients, but these columns are necessary for certain tests that we may wish to apply.

The most striking feature of the analysis so far is the wide differences between the correlations for the station means and the variety means. Also, there is an evident relation between the total nitrogen and saccharifying activity as the total nitrogen changes from station to station. Although the station correlation is represented by only 10 degrees of freedom, it is highly significant. The variety correlation is quite insignificant. This indicates that genetic factors that cause differences between the varieties in the total nitrogen content of the grain do not have a corresponding effect on the saccharifying activity of the malt extract. This is an excellent example of the heterogeneity of the covariance and illustrates clearly the necessity for the separation of the total covariance into its component parts.

The next point to clear up in this study is the variation in the regressions for individual varieties. We know that when the varieties are considered as a group, the correlation between the two variables is high. This is indicated by the value of the coefficient for the station means. This correlation may be significantly higher for some varieties than for others, and this will be important information for us to have in evaluating the varieties. A test of the significance of the heterogeneity of the variety regressions can be carried out very easily by means of an extension of the covariance analysis.

The first step is to calculate the sums of squares and products for each variety. These are given in Table II.

TABLE II  
ANALYSIS OF COVARIANCE FOR TESTING HETEROGENEITY OF VARIETY REGRESSIONS

Variety	$\Sigma(x^2)$	$\Sigma(xy)$	$\Sigma(y^2)$	DF	Regression	Residual	DF
1	1.1498	1.7903	2.9745	11	2.7876	0.1869	10
2	1.8104	0.9613	0.8930	11	0.5104	0.3826	10
3	1.6141	1.8985	3.9864	11	2.2330	1.7534	10
4	1.2609	1.9195	3.2895	11	2.9221	0.3674	10
5	1.5873	1.7014	2.1109	11	1.8237	0.2872	10
6	1.7591	2.6461	4.6605	11	3.9804	0.6801	10
7	1.6923	2.7206	4.7231	11	4.3737	0.3494	10
8	1.8202	2.9503	5.1203	11	4.7820	0.3383	10
9	1.7302	1.5772	1.7513	11	1.4377	0.3136	10
10	2.0446	3.4896	6.6857	11	5.9558	0.7299	10
11	1.3582	1.1139	1.5959	11	0.9135	0.6824	10
12	1.4622	1.1139	1.2111	11	0.8486	0.3625	10
Column totals	19.2893	23.8826	39.0022	132		6.4337	120
All varieties	19.2893	23.8826	39.0022	132	29.5697	9.4325	131

In each line including the last, the regression sum of squares is calculated as in Table I. The test of significance is finally as follows—

	Residual	DF	Mean square	F	5% pt
All varieties	9.4325	131			
Within varieties	6.4337	120	0.05361		
Difference	2.9988	11	0.2726	5.08	1.86

The F value is high and definitely establishes the significance of differences among the regressions for the varieties.

There are other procedures and tests of significance that flow from the covariance analysis, but those described in this example are undoubtedly of the greatest importance for experiments of this type. We shall now discuss a type of experiment in which the covariance analysis is applied in a somewhat different manner.

### Subdivision of Treatment Components

A fairly common type of experiment is one in which a number of treatments are compared at two or more levels of a second factor. For example, we might have a series of flours of different varieties that are to be tested for loaf volume with the addition of different amounts of potassium bromate. A study of published results indicates that a method of analysis particularly appropriate for data from experiments of this kind is rarely used. A greater knowledge of this method would seem to be worth while in the extraction of the maximum amount of information from the experiment, and it also seems likely that a knowledge of the technique of analysis would be of assistance in working out the design. Foreknowledge of what one is going to do with data is bound to have some influence on how the experiment is laid out.

In order to demonstrate the method of analysis it will be necessary to illustrate the procedure known in statistics as the splitting up of degrees of freedom into orthogonal components. If we have three determinations that we shall designate by A, B, and C, the differences between these three determinations are represented by two degrees of freedom. The two degrees of freedom can be split up into orthogonal components. In the sense in which it is used here, the word "orthogonal" means independent. In other words, one of the components can have any value whatever without its having any effect on the value of the other component. One of the components can be defined arbitrarily, but when the first one is defined the second one is fixed by rule. Three simple ways of defining one component would be to take the difference between any two of the determinations such as  $C - A$ . The rule for the derivation of the second component is best illustrated

by setting up the first comparison as follows:

A	B	C
-1	0	+1

The numbers are referred to as coefficients and for any such comparison the sum of these coefficients must be zero. The second and independent comparison must be built up so that the sum of the coefficients is zero and also the sum of the products of the coefficients for the two comparisons. The second comparison can now be set up below the first.

A	B	C
-1	0	+1
+1	-2	+1

On examining the coefficients in each line we note that they conform with the rule that their sums and sums of products must be zero.

The next step is to calculate the sums of squares for the two components. The first one is given by  $(C - A)^2/2$ , and the second by  $(A + C - 2B)^2/6$ , where the rule for the divisor is to take the sum of the squares of the coefficients. If we add the above two sums of squares we will get the sum of squares for the two degrees of freedom calculated in the ordinary way. This can be used as a check on the calculations.

It should be obvious that the procedure of splitting up the degrees of freedom and the corresponding sums of squares may have a very definite logical basis. This is particularly true if A, B, and C represent three levels of one factor as in an experiment on loaf volume in which the levels are 1, 2, and 3 mg of bromate added to the dough. In such an experiment some sort of trend in the results would be expected, and it would be logical to compare the volume for the smallest quantity of bromate with the volume for the largest amount of bromate added. Actually the method being used here is a short cut to analyses by means of regression and correlation coefficients. The effect measured by  $C - A$  is actually the same as that which would be measured by a regression straight line fitted to the three points, in which the level of bromate is the independent variable and loaf volume the dependent variable. In terms of regression analysis the sum of squares calculated as above for  $C - A$  is  $[\Sigma(xy)]^2/\Sigma(x^2)$ , where  $x$  and  $y$  are measured from their means,  $x$  being the independent variable and  $y$  the dependent variable. This is the portion of the total sum of squares of  $y$  that is accounted for by the regression straight line. The single degree of freedom for  $C - A$  represents the linear regression function, and the

single degree of freedom for  $A + C - 2B$  represents the additional constant involved in fitting a quadratic function of the form  $Y = a + bx + cx^2$ .

We should notice in the first place that there are three different ways in which the two degrees of freedom can be split up into linear and quadratic components. This will depend entirely on the choice of the experimenter, but in an example such as the one mentioned above, the linear component will usually be  $C - A$ . The most logical division, however, will arise from the general characteristics of the experiment. Suppose that  $A$  represents a zero level of some treatment and  $B$  and  $C$  are the two actual levels. One of the most interesting comparisons is therefore  $B + C - 2A$ , which is equivalent to comparing treatment with no treatment.

If there are more than three levels of the one factor, and these are to be split up into orthogonal degrees of freedom having some logical value for the experiment, we can proceed in a similar manner. For example, with four levels represented by  $A$ ,  $B$ ,  $C$ , and  $D$ , the linear quadratic and cubic components are as follows:

A	B	C	D	
+3	+1	-1	-3	Linear component
-1	+1	+1	-1	Quadratic component
+1	-3	+3	-1	Cubic component

It is only necessary to have the coefficients and any similar problem can be solved. These are given in the tables by Fisher and Yates (1938) for fitting components up to the 5th degree for any number of levels up to 52. The divisors for obtaining the sums of squares are also given in these tables and this saves a good deal of labor when working with five or more levels.

A different method of splitting up the degrees of freedom is indicated in certain examples. Suppose that  $A$  and  $B$  are two levels of one improver and  $C$  and  $D$  are two levels of another improver. Here, two comparisons are obvious, and then the third comparison will be fixed. The three comparisons are as follows:

A	B	C	D
-1	-1	+1	+1
-1	+1	-1	+1
-1	+1	+1	-1

The first comparison is between the two kinds of improvers. The

second is between the two levels for both improvers. The third represents the extent to which the improvers give different results at the two levels, and is comparable therefore to an interaction between improvers and levels.

With this brief discussion of methods of splitting up degrees of freedom into logical components, we are ready to consider the analysis of a hypothetical experiment. Let us suppose that five flours are tested with four levels of bromate added; for example, with 1, 2, 3, and 4 mg. The data could be arranged in the form of a table as follows, and for each variety we shall determine the totals, the linear components, the quadratic components, and the cubic components.

Flours	Mg bromate				Variety totals	Linear components	Quadratic components	Cubic components
	1	2	3	4				
A								
B								
C								
D								
E								
Totals								

The analysis of variance can now be outlined. It will be noted that the analysis differs from a simple analysis in that the treatment sum of squares and the interaction sum of squares are broken up into

#### OUTLINE OF ANALYSIS OF VARIANCE

	DF	Mean square
Varieties	4	$v$
Treatments—linear effects	1	1
—quadratic effects	1	$q$
—cubic effects	1	$c$
Interaction—linear effects $\times$ varieties	4	$1 \times v$
—quadratic effects $\times$ varieties	4	$q \times v$
—cubic effects $\times$ varieties	4	$c \times v$

appropriate linear, quadratic, and cubic components. In a simple analysis, the interaction mean square would be used to test the significance of the mean squares for varieties and treatments. In the above form the mean square for the interaction can likewise be used to test the three components of the treatment effect. The results will show whether or not there is a tendency towards a definite type of trend. It is possible, in addition, to make a test of the linear and quadratic portions of the interaction. This is done by performing two supplementary tests, setting up an analysis for each. These are as follows:

	DF	MS	F
Linear effect $\times$ varieties	4		
$q + c + (q \times v) + (c \times v)$	10		
	DF	MS	F
Quadratic effect $\times$ varieties	4		
$c + (c \times v)$	5		

The general procedure followed in these supplementary analyses is the same as in the ordinary covariance analysis; except that in the first place the procedure here is shown in relation to the sums of squares and degrees of freedom of the ordinary analysis of variance, and in the second place it is carried forward past the fitting of linear regressions to the fitting of second and third degree constants to the results for the treatments. If we were dealing with the linear effects only, we would have divided the treatment degrees of freedom into two portions; linear regression and deviations from linear regression, for 1 and 2 degrees of freedom, respectively. The interaction would have been divided correspondingly and we would have carried out only the first of the supplementary tests mentioned above.

It is important to note the exact relation of the supplementary tests made here to the tests for heterogeneity of regression applied in the covariance analysis. The first test of the interaction of the linear effects with varieties is a test of the heterogeneity of the linear regres-

TABLE III

LOAF VOLUMES FOR 17 VARIETIES OF WHEAT TESTED AT FOUR LEVELS OF POTASSIUM BROMATE ADDED IN THE BAKING PROCEDURE

Variety	Levels of bromate in mg				Total	Lin. comp.	Quad. comp.	Cu. comp.
	1	2	3	4				
1	10.8	10.6	9.8	8.8	40.0	-6.8	-0.8	0.4
2	9.8	9.6	8.6	8.2	36.2	-5.8	-0.2	1.4
3	8.5	8.2	7.7	7.4	31.8	-3.8	0	0.4
4	8.2	7.6	7.2	7.0	30.0	-4.0	0.4	0
5	10.4	10.6	9.8	9.4	40.2	-3.8	-0.6	1.4
6	9.6	9.8	9.2	8.4	37.0	-4.2	-1.0	0.6
7	9.0	9.0	8.8	7.8	34.6	-3.8	-1.0	-0.6
8	8.6	8.7	8.5	8.5	34.3	-0.5	-0.1	0.5
9	9.4	10.0	9.6	9.6	38.6	0.2	-0.6	1.4
10	10.0	10.2	9.6	9.0	38.8	-3.6	-0.8	0.8
11	9.4	9.6	9.5	9.2	37.7	-0.7	-0.5	0.1
12	8.4	8.7	8.8	8.8	34.7	1.3	-0.3	0.1
13	6.6	6.5	6.8	6.6	26.5	0.3	-0.1	-0.9
14	9.1	8.9	8.4	7.8	34.2	-4.4	-0.4	0.2
15	10.8	10.7	10.2	10.0	41.7	-2.9	-0.1	0.7
16	7.5	7.4	7.2	7.2	29.3	-1.1	0.1	0.3
17	8.4	8.6	8.2	8.2	33.4	-1.0	-0.2	1.0
Totals	154.5	154.7	147.9	141.9	599.0	-44.6	-6.2	7.8



sion coefficients. Then, by a simple extension of the procedure, we test the heterogeneity of the quadratic regressions. This process can be carried forward indefinitely. If the number of levels is greater than four, we may, for example, wish to test the heterogeneity of the cubic regressions. With a still greater number of levels it would be possible to make further tests, but it is not often that we will be interested in effects higher than those of the third degree.

We shall now apply the above method to a set of data obtained by Larmour (1941) on the reactions of a series of flours from 17 varieties that were baked with four levels of potassium bromate. These data are given in Table III together with a portion of the calculations. In the actual experiment another treatment was included in which no bromate was added. This level is omitted here in order to simplify the example. The loaf volumes, which were given by Larmour in cc, have here been coded by dividing by 100 and rounding off the last decimal figure. Linear, quadratic, and cubic components have been calculated for each variety and are given in the last three columns of the table.

In the first place, the usual analysis is set up giving the sums of squares of varieties, treatments, and interaction. This works out as follows:

	Degrees of freedom	Sums of squares	Mean square	F	5% point
Varieties	16	70.0097	4.376	40.1	1.86
Treatments	3	6.5947	2.198	20.2	2.80
Interaction	48	5.2303	0.1090		

The next step is to split up the treatment sum of squares into linear, quadratic, and cubic components. These are calculated from the treatment totals of Table III.

Linear component

$$= \frac{(-3 \times 154.5 - 1 \times 154.7 + 1 \times 147.9 + 3 \times 141.9)^2}{20 \times 17} = 5.8505$$

Quadratic component

$$= \frac{(1 \times 154.5 - 1 \times 154.7 - 1 \times 147.9 + 1 \times 141.9)^2}{4 \times 17} = 0.5653$$

Cubic component

$$= \frac{(-1 \times 154.5 + 3 \times 154.7 - 3 \times 147.9 + 1 \times 141.9)^2}{20 \times 17} = 0.1789$$

The coefficients used in the above equations can be obtained from the tables by Fisher and Yates (1938).

To determine the corresponding interactions we deal with the figures in the three columns on the right of Table III. Thus:

Linear effects  $\times$  varieties

$$= [(6.8^2 + 5.8^2 + \dots + 1.0^2)/20] - 5.8505 = 4.2665$$

Quadratic effects  $\times$  varieties

$$= [(0.8^2 + 0.2^2 + \dots + 0.2^2)/4] - 0.5653 = 0.6297$$

Cubic effects  $\times$  varieties

$$= [(0.4^2 + 1.4^2 + \dots + 1.0^2)/20] - 0.1789 = 0.3341$$

Note that the correction terms are the components previously calculated in dividing up the sums of squares for treatments. The complete analysis can now be set up. The tests of significance emphasize that

	DF	SS	MS	F	5% point of F
Linear effect	1	5.8505	5.8505	53.7	4.04
Quadratic effect	1	0.5653	0.5653	5.19	4.04
Cubic effect	1	0.1789	0.1789	1.64	4.04
Interaction	48	5.2303	0.1090		

the large differences between the treatments are mainly linear effects; in other words, there is a definite tendency, when all varieties are combined, for the quantities of bromate added to bring about a proportionate reduction in the loaf volumes. However, the quadratic effect is also significant; and on examining the totals for the treatments, we note that there is a slight increase in volume from level one to level two and a definite falling off of the volumes for the third and fourth levels. The cubic effect is insignificant, and this is exactly what we would expect from an examination of the treatment totals.

In view of the fact that the linear and quadratic effects are significant, it is of interest to examine the components of the interaction. We can make two simple tests as follows:

	DF	SS	MS	F	5% point of F
Lin. $\times$ varieties	16	4.2665	0.2666	5.31	1.95
Error	34	1.7080	0.05024		
Quad. $\times$ varieties	16	0.6297	0.03936	1.30	2.29
Error	17	0.5130	0.03018		

In order to obtain the error for testing the linear interaction effect, we use all the sums of squares representing deviations from linear regression. We have, therefore,  $0.5653 + 0.1789 + 0.6297 + 0.3341 = 1.7080$ . Similarly the error for testing the quadratic interaction effect contains all the sums of squares representing deviations from quadratic regression. This is  $0.1789 + 0.3341 = 0.5130$ .

These tests have furnished information of value. They have shown that, although the linear effect for all varieties combined is significant, the linear effects for the varieties taken individually are not uniform. It is not possible, however, to detect any differences between the quadratic effects.

We can again show that what we have done in the last two tests is exactly the same as is done in the covariance analysis when testing the heterogeneity of regressions. The method for setting up the analysis of covariance is as follows:

Total SS	DF	Linear regression SS	DF	Residual	DF	Mean square	F	5% point
11.8250	51	10.1170	17	1.7080	34			
11.8250	51	5.8505	1	5.9745	50			
Error Heterogeneity of regressions				1.7080 4.2665	34 16	0.05024 0.2666	5.31	1.95

In a similar manner we can set up the covariance analysis for the quadratic effects.

Total SS	DF	Quadratic regression SS	DF	Residual	DF	Mean square	F	5% point
1.7080	34	1.1950	17	0.5130	17			
1.7080	34	0.5653	1	1.1427	33			
Error Heterogeneity quad. regression				0.5130 0.6297	17 16	0.03018 0.03936	1.30	2.29

### Selection of Levels

If it is agreed that the analytical methods demonstrated in the last example are useful and practical, a further point can be made in connection with experimental design. Since the simplicity of the method arises chiefly from the fact that the levels of the factor being studied are in arithmetical progression, it is suggested that time and effort can be saved by careful attention to the levels used. It happens frequently that the experiment calls for the use of levels that are not in arithmetical progression, and the experimenter may inadvertently use a series of levels that are not in any sort of mathematical progression. This is very likely to make the calculations in any method of analysis much more difficult, and it is particularly true with the method of analysis described above.

Let us suppose for example that the dependent variable being studied is expected to react as indicated by the following hypothetical data.

Levels ( $x$ )	0.10	0.25	1.00	5.00	20.00
Determinations ( $y$ )	15.0	15.3	15.7	15.6	16.0

The levels here are of the kind that are sometimes arbitrarily selected without giving much thought to methods of analysis. At low levels of  $x$ , much smaller increases in  $x$  are required to produce a given increase in  $y$  than at higher levels of  $x$ . The obvious procedure in this example is to set up levels such that their logarithms are in arithmetical progression. We could use the levels 0.10, 0.40, 1.6, 6.4, 25.0. The hypothesis that there is a linear relationship between values of  $y$  and the logarithms of  $x$  could then be tested in a very simple manner by replacing the values of  $x$  by 1, 2, 3, 4, 5. Most experiments of this type are for the purpose of testing a hypothesis of this kind. There are a great variety of hypotheses that can be tested, but, generally speaking, these can be expressed as some form of mathematical relation, and the levels set up can be such that they may be transformed readily to a series of natural numbers. Actually there is no excuse for a series of levels that do not form a regular series of some sort. With this in mind it should be possible to make the statistical work on many problems much easier than would otherwise be the case.

### Summary

There is a very close relation between methods of analysis and experimental design. A study of different methods of analysis may suggest new designs or changes in others.

A description of a simple covariance analysis is given, with the object of demonstrating how the method can be used to advantage in certain types of experiments.

The procedure of splitting up degrees of freedom in the analysis of variance is described, and an example given showing how the maximum amount of information can be extracted from an experiment in which one of the factors is tested at various levels.

Reference is made to the possibility of simplifying calculations by being careful in the selection of levels in a factorial experiment.

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# EFFECT OF ENVIRONMENT DURING THE GROWTH AND DEVELOPMENT OF WHEAT ON THE BAKING PROPERTIES OF ITS FLOUR<sup>1</sup>

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Recent investigations have shown that there is a linear relation between protein content and loaf volume *within* wheat varieties and a variation in baking quality *between* varieties (Aitken and Geddes, 1934 and 1939; Larmour, 1931; Larmour, Working, and Ofelt, 1939 and 1940; McCalla, 1940; Bayfield, Working, and Harris, 1941; and Johnson, Swanson, and Bayfield, 1943).<sup>3</sup> The exceedingly high correlations between protein and volume obtained in the more recent work indicate that within varieties there was little variation in quality, practically the entire variation being in the amount of protein. Accordingly, since differences in protein content are due largely to differences in the environments under which the wheats are grown (McCalla and Rose, 1941), one obtains the impression that environment has little effect on quality. However, it should be noted that much of this work was done on composite samples, the composites representing a number of localities which produced similar protein content, or the results were reported as the averages of loaf volumes for each increment of protein content. Marked environmental effects occurring in a few localities or small effects in a number of localities might be largely eliminated by these procedures.

Sandstedt and Ofelt (1940), by diluting flours to a given protein content with starch, showed that within a variety the baking quality seemed to decrease with an increase in protein content. However, they pointed out that this was not true for all varieties and that high-protein samples grown in one particular locality had outstanding quality. This suggested that the quality of these high-protein flours was due to the environment under which they were grown.

The purposes of the present paper are to offer further evidence of the considerable differences in baking properties which may be obtained by growing wheat in different localities and to show that the baking quality of flour may be materially dependent on the conditions under which the wheat is grown.

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<sup>3</sup> Some seeming contradiction arises owing to the quite common use of *protein content* as a measure of *quality* in wheat. For example, McCalla and Rose (1941) concluded that environment was more effective in determining *quality* of wheat than was *variety*.

### Materials and Methods

*Wheat Origin and Characteristics.* The Agronomy Department of the Nebraska Experiment Station, the Agricultural Extension Service, and the Nebraska Grain Improvement Association each year conduct regional performance tests of promising new strains in comparison with standard varieties. These tests are so planned that all varieties grown in one locality are comparable. In the fall of 1939, 22 such tests were planted. Because of an exceedingly dry fall in the south central part of the state with the consequent poor germination and severe winter-killing, only 14 of these were harvested, as shown in Figure 1. The

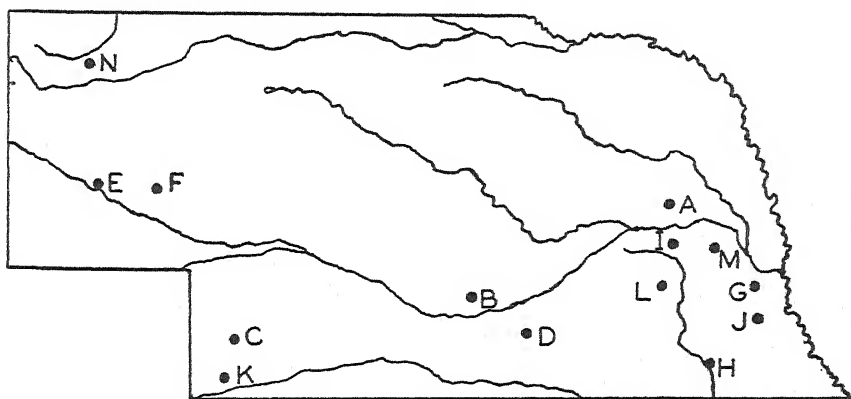


Fig. 1. Location of cooperative wheat test plots—1940.

grain from six varieties included uniformly in all of the tests provides the basis for the studies herein reported.

In order to show the relation of protein to loaf volume to the best advantage, the test localities are designated by letters; the locality which produced the lowest average protein content is designated as *A*, that producing the next higher average protein as *B*, etc. These locality designations apply to all varieties regardless of their individual protein content.

The yields and test weights (Table I) of the wheats and the protein content of the flours from the wheats (Table II) are presented as indications of the wide variation in the environmental conditions prevailing during the 1939-1940 growing season, causing a range in average yield from 13.6 bu at *N* to 38.3 bu at *B*, and from 12.4% protein in the flour at *A* to 16.9% at *N*. However, such wide variations may be expected in the Great Plains region. Nebraska, itself, has a considerable range both in soil type and in climate. The test weights may be considered as indicative of the condition of the grain and of its approximate market grade.

TABLE I

COMPARATIVE YIELDS AND TEST WEIGHTS OF WINTER WHEAT VARIETIES IN COOPERATIVE VARIETY DEMONSTRATION PLOTS, 1940<sup>1</sup>

Locality	Mean of all varieties		Variety	Mean of all localities	
	Yield	Test weight		Yield	Test weight
	<i>bu/acre</i>	<i>lb/bu</i>		<i>bu/acre</i>	<i>lb/bu</i>
A	34.3	60.1	Turkey	22.1	58.3
B	38.3	61.5	Nebred	23.3	59.5
C	34.4	61.0	Tenmarq	24.4	57.5
D	21.6	54.4	Cheyenne	24.4	59.4
E	23.7	59.0	Blackhull	24.9	59.8
F	16.3	58.1	Chiefkan	26.0	60.0
G	22.0	59.2			
H	29.4	59.9			
I	26.9	59.8			
J	23.0	60.2			
K	18.2	59.6			
L	17.4	59.9			
M	19.0	59.8			
N	13.6	55.0			

<sup>1</sup> "Results of the 1940 cooperative small grain variety demonstration trials," G. T. Webster, D. L. Gross, and T. A. Kieselbach.

**Milling.** The wheats were milled to 85% patent flours on a four-stand Allis-Chalmers experimental mill.

**Baking Method.** The micro-baking technique described by Van Scoyk (1939) as modified by Sandstedt and Ofelt (1940) was used. The baking formula was as follows: 25 g of flour (15% moisture basis), 3% yeast, 6% sugar, 1% salt, 3% shortening, 0.2% malt flour, and 0.001% potassium bromate. Each dough was given *optimum* mixing in a National micromixer. The optimum, which is essentially *development to optimum consistency*, was determined by observing the transition of the dough during mixing from the rough appearance of an undermixed dough to the "velvety smoothness" of a well-developed dough. Proofing was to 6.5 cm height in the tall form micro pans. Flours of the same variety from all localities were baked on one day. This bake was repeated on another day; accordingly, each flour was baked in duplicate, the duplicates being baked on different days. Using this order of baking, differences in results owing to locality become highly significant, especially if the locality effect tends to be similar for all varieties.

The precision which may be attained by an experienced baker when using this experimental baking procedure is shown by the analysis of variance given in Tables III, IV, V, and VI. For testing the variability resulting from the baking procedure itself, the mean square of the remainder was used as error. Calculated on this basis the error of

the difference between two flours (each baked in duplicate) was 6.5 sec in mixing time and 3.2 cc in volume. Accordingly, the differences required for significance (based on the 5% point) would be 20 sec and 10 cc.

Sandstedt and Ofelt (1940) proposed that experimental flours of varying protein content be diluted to a common protein basis for quality determination. Accordingly, each flour was diluted with wheat starch to an artificial 10% protein content and again baked in duplicate, using the same formula and baking procedure as used for the original flours. The starch used for dilution was prepared in the laboratory, discarding the "amyloextrin" fraction (Sandstedt, Jolitz, and Blish, 1939).

*Analysis of Variance.* The analysis of variance was made according to procedures outlined by Snedecor (1937). All calculations were based on the original determinations (mixing times and loaf volumes), not on the averages of duplicates as given in the tables. As already stated, the mean square of the remainder was used as the error for calculating the standard deviation and error of difference between two flours in showing the precision of the baking procedure. It was also used for testing the significance of the variety  $\times$  locality interaction. However, as it was desired to test for significant differences between localities considering all varieties, the mean square of the interaction  $V \times L$  must be used as error as this value is shown to be significant. Accordingly, as given in the tables, the error of the difference between two flours and the difference required for significance of the varietal and locality means are calculated on this basis.

*Definition of Quality.* Larmour, Working, and Ofelt (1939) defined baking quality as the capacity of a flour to fulfill the loaf volume prediction made on the basis of its protein content. Quality when used in this paper is limited to the above definition.

### Experimental Results and Discussion

*Flour Protein.* As previously stated, the range in protein content of the flours (Table II) indicates a wide variation between the environments under which the wheats were grown. The Turkey and Black-hull wheats were slightly higher in protein than the other varieties, but there was no consistent tendency for one variety to produce significantly more or less protein than another. The differences between varieties were slight compared to the differences between localities. Similar results were reported by McCalla and Rose (1941) and by a number of earlier investigators. Bailey (1925) gives a comprehensive review of the extensive literature on the influence of environment on the *composition* of wheat.



TABLE II  
EFFECT OF ENVIRONMENT AND VARIETY ON FLOUR PROTEIN CONTENT<sup>1</sup>

Locality	Flour protein content (15% moisture basis)						
	Turkey	Nebred	Tenmarq	Cheyenne	Blackhull	Chiefkan	Mean
	%	%	%	%	%	%	%
A	11.6	12.5	12.2	12.5	12.7	12.8	12.4
B	13.2	12.5	11.4	12.4	13.2	12.2	12.5
C	13.1	12.5	12.8	13.1	13.6	13.2	13.0
D	14.3	13.1	13.6	13.3	13.7	13.1	13.5
E	14.6	14.1	14.2	13.8	14.4	13.9	14.2
F	15.3	15.0	14.7	14.8	14.7	14.6	14.8
G	15.8	14.9	14.8	13.9	16.4	15.1	15.1
H	15.2	15.0	15.9	15.2	15.4	14.4	15.2
I	16.0	15.3	15.3	15.4	15.3	14.7	15.3
J	15.4	15.8	15.6	15.2	15.3	15.4	15.4
K	15.9	15.5	15.8	15.6	15.5	15.0	15.5
L	15.7	15.4	15.7	15.2	15.8	15.3	15.5
M	16.9	16.9	16.3	15.3	15.8	16.0	16.2
N	17.4	17.5	16.7	16.7	16.5	16.5	16.9
Mean	15.0	14.7	14.6	14.4	14.9	14.4	

<sup>1</sup> Cooperative wheat tests (1940).

*Absorption.* In baking these samples it soon became apparent that there were a number of flour characteristics which varied with changes in environment and that in most cases the varieties tended to respond in a similar manner to any particular change in environment, *e.g.*, all flours from locality *N* had unusually high absorptions; 2 to 4% higher than when grown in other localities which produced a similar quantity of protein. Locality *N* produced the highest protein wheats, but the difference in protein quantity between localities *M* and *N* was not great enough to account for the difference in absorption.

*Handling Properties.* The handling properties of the doughs from wheats grown at *A* varied considerably from the handling properties of the same varieties grown in the other localities. The flours from this locality had a tendency to produce soft and somewhat sticky doughs. This was particularly evident when comparing localities *A* and *B*, which produced flours with similar protein content.

*Mixing Requirements.* The mixing requirements of the doughs, the time required to reach *optimum development*, varied markedly with environment. This is shown by the data and analysis of variance presented in Tables III and IV. The mixing requirements are given as the time in minutes required to reach optimum consistency; each figure being the average of duplicate mixes. The average mixing time for the series of flours with natural protein content was 2 min 17 sec; that for those diluted to 10% protein content was 2 min 23 sec.

TABLE III  
EFFECTS OF ENVIRONMENT AND VARIETY ON MIXING REQUIREMENT OF FLOURS  
(Optimum mixing time—average of duplicates)

Locality	Turkey	Nebred	Tenmarq	Cheyenne	Blackhull	Chiefkan	Mean
	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>
A	2.45	2.38	2.09	3.96	1.58	1.54	2.33
B	1.92	2.92	2.38	2.92	1.50	1.54	2.19
C	1.79	2.13	1.71	3.54	1.38	1.38	1.99
D	2.67	4.42	2.71	4.75	1.83	1.71	3.01
E	1.88	2.79	1.96	3.13	1.67	1.50	2.15
F	2.00	3.13	1.96	3.79	1.58	1.54	2.33
G	1.75	2.84	2.30	4.05	1.50	1.67	2.35
H	1.79	2.75	2.04	3.63	1.54	1.54	2.21
I	1.63	2.38	1.88	2.79	1.46	1.38	1.92
J	1.67	2.46	2.00	3.42	1.46	1.50	2.08
K	1.63	1.92	1.48	2.46	1.42	1.33	1.71
L	1.88	2.92	2.25	3.92	1.67	1.42	2.34
M	1.75	2.58	2.04	2.88	1.46	1.42	2.02
N	2.67	4.17	3.25	5.13	2.42	1.80	3.24
Mean	1.96	2.84	2.15	3.60	1.61	1.51	
Correlation coefficient, protein X mixing time	0.20	0.12	0.08	0.04	0.24	0.10	

Difference Required for Significance of Protein-Mix time:  $r = 0.53$

#### ANALYSIS OF VARIANCE

Factor	Degrees of freedom	Mean square	F	
Variety	5	17.95	95.0**	Error of difference between two
Locality	13	1.98	10.5**	flours = 0.309
Interaction	65	0.189 <sup>1</sup>	7.98**	Based on the 5% point, difference
V X L				required for significance of:
Remainder	84	0.0237		Varietal means = 0.233
Total	167			Locality means = 0.355

<sup>1</sup> Used as error.

\*\* Significant at 1% point.

The significance of differences in mixing time is indicated by the analysis of variance. Differences between locality averages greater than 21 sec, and between variety averages greater than 14 sec (differences required for significance based on the 5% point) may be considered as significant.

All varieties grown at locality *K* required a short mixing time, while those grown at *N* required a long mixing. Chiefkan (noted for its short mixing requirement) from *N* required as much mixing as Turkey or Tenmarq from *K*. Blackhull grown at *N* required more mixing than these other varieties grown at *K* and even as much as Cheyenne (normally requiring an exceedingly long mix) grown at *K*. It is interesting, though perhaps to be expected, that the difference between

TABLE IV

EFFECT OF ENVIRONMENT AND VARIETY ON MIXING REQUIREMENTS OF FLOURS  
(Diluted to 10% protein content)  
(Optimum mixing time—average of duplicates)

Locality	Turkey	Nebred	Tenmarq	Cheyenne	Blackhull	Chiefkan	Mean
	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>
A	2.46	2.17	2.08	3.92	1.71	1.54	2.31
B	1.79	3.13	2.46	3.00	1.54	1.59	2.25
C	1.80	2.25	2.00	3.54	1.46	1.50	2.09
D	2.46	3.95	2.71	5.42	1.83	1.79	3.03
E	1.79	2.88	2.00	3.59	1.75	1.50	2.24
F	1.88	3.13	2.25	4.29	1.63	1.71	2.48
G	1.75	2.88	2.46	4.71	1.50	1.75	2.51
H	1.63	2.79	2.33	3.67	1.50	1.67	2.26
I	1.72	2.42	1.92	3.21	1.46	1.46	2.02
J	1.58	2.58	2.17	3.92	1.46	1.58	2.22
K	1.71	2.00	1.58	2.63	1.38	1.42	1.78
L	1.75	2.96	2.25	4.21	1.80	1.75	2.45
M	1.75	2.84	2.25	2.92	1.75	1.46	2.16
N	2.58	4.50	3.59	5.84	2.42	1.96	3.48
Mean	1.89	2.89	2.29	3.92	1.66	1.62	

## ANALYSIS OF VARIANCE

Factor	Degrees of freedom	Mean square	F	
Variety	5	22.25	86.6**	Error of difference between two
Locality	13	2.18	8.48**	flours = 0.359
Interaction	65	0.257 <sup>1</sup>	26.5**	Based on the 5% point, difference
V × L				required for significance of:
Remainder	84	0.00969		Varietal means = 0.272
Total	167			Locality means = 0.414

<sup>1</sup> Used as error.

\*\* Significant at 1% point.

varieties is less in the localities requiring short mixing times and greater in localities requiring long mixing times; the difference between Chiefkan and Cheyenne from K was only 1.1 min, while at N the difference was 3.4 min. Also, the difference between localities was less for varieties having short mixing requirements and greater for varieties having long mixing requirements; the difference between Chiefkan wheats from K and N was 0.5 min, while for Cheyenne it was 2.7 min.

*Relation of Protein Content to Mixing Time.* The mixing requirements of the diluted flours (Table IV) are fairly good checks of the mixing requirements of the corresponding flours of natural protein content. This is strong evidence that protein content does not affect the mixing requirement as determined by this method. Further substantiation is obtained from the correlation between the mixing requirement and protein content for each variety. These correlation

coefficients ranging from .04 to .24 are given in Table III. They are not significant. It is quite evident that the protein content had little if any effect on the mixing requirement. These data substantiate conclusions drawn from mixogram studies on flours, and on flours diluted with starch to various protein levels, by Ofelt and Sandstedt (1941) and by Johnson, Swanson, and Bayfield (1943).

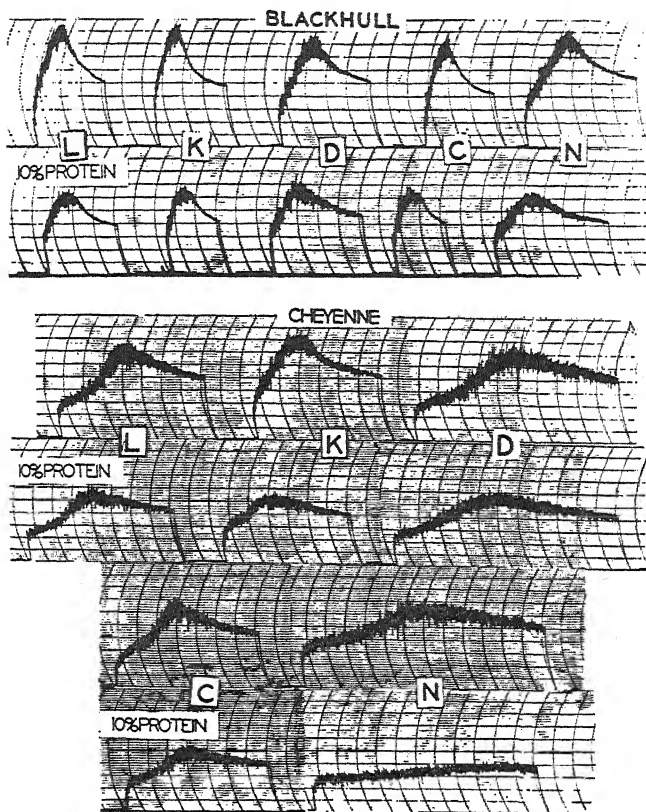


Fig. 2. Effect of environment on type of mixograms produced by Blackhull and Cheyenne wheat flours. Upper curves were made with flours of natural protein content; lower curves with the flours diluted to 10% protein level.

**Mixograms.** The differences in mixing characteristics of flours from various localities may be shown quite effectively by means of mixograms (recording dough mixer curves). All mixograms of this series of flours were made using the *baking absorptions*, i.e., the absorptions determined for use in the baking procedure. Cheyenne and Blackhull are representative of varieties requiring exceedingly long and short mixing. The mixograms for these varieties grown in environments L, K, D, C, and N (Figure 2) are used to illustrate the differences in

mixing characteristics which may be produced by environment in flours of the same variety with nearly equal protein content, the pairs *L K* and *D C* being comparable (Table II). The upper curves were obtained from the flours with their natural protein content, while the lower curves were obtained from the flours diluted to 10% protein with wheat starch. Dilution to a 10% protein content for determining curve characteristics makes all curves directly comparable (Ofelt and Sandstedt, 1941).

While in general the curves for the two varieties were typical, the environment produced, in each variety, a wide range in curve character with the curve for Blackhull grown at *N* tending to approach in character the curve for Cheyenne grown at *K*. Swanson (1939) showed

TABLE V  
EFFECT OF ENVIRONMENT AND VARIETY ON LOAF VOLUME  
(Loaf volume—average of duplicates)

Locality	Turkey	Nebred	Tenmarq	Cheyenne	Blackhull	Chiefkan	Mean
A	161	164	170	168	169	154	164
B	158	160	160	156	155	138	155
C	165	164	169	165	162	145	162
D	207	209	203	189	184	154	191
E	163	170	172	168	159	145	163
F	166	184	186	168	162	142	168
G	182	204	197	189	175	159	184
H	180	206	191	181	170	154	180
I	186	217	199	190	175	157	187
J	199	219	208	201	185	163	196
K	173	193	175	167	164	142	169
L	200	218	219	185	175	153	192
M	201	229	218	197	169	155	195
N	188	204	212	171	185	152	184
Mean	181	196	192	178	171	151	
Regression coefficient, loaf volume on protein content	6.18	11.0	9.10	4.13	4.0	3.1	

#### ANALYSIS OF VARIANCE

Factor	Degrees of freedom	Mean square	F	
Variety	5	7233	48.7**	Error of difference between two flours = 8.6 cc
Locality	13	2357	15.9**	Based on the 5% point, difference required for significance of:
Interaction	65	148 <sup>1</sup>	7**	Varietal means = 6.5 cc
V × L	84	20.0		Locality means = 9.9 cc
Remainder	167			
Total				

<sup>1</sup> Used as error.  
\*\* Significant at the 1% point.

TABLE VI  
EFFECT OF ENVIRONMENT AND VARIETY ON LOAF VOLUME OF FLOURS  
(Diluted to 10% protein content)

Locality	Turkey	Nebred	Tenmarq	Cheyenne	Blackhull	Chiefkan	Mean
	cc	cc	cc	cc	cc	cc	cc
A	159	142	143	144	140	138	144
B	147	143	142	142	130	124	138
C	152	141	139	137	137	128	139
D	162	164	153	154	141	138	152
E	142	137	137	137	125	126	134
F	139	138	137	132	128	120	132
G	148	147	143	140	129	129	139
H	144	146	139	133	131	125	136
I	145	145	141	135	132	123	137
J	149	145	144	136	136	128	140
K	135	134	129	117	118	115	125
L	145	149	144	133	127	126	137
M	142	145	142	132	127	119	135
N	139	134	141	119	131	121	131
Mean	146	144	141	135	131	126	
Protein-volume regression coefficient	-3.18	-1.30	-0.70	-5.5	-2.26	-2.76	

## ANALYSIS OF VARIANCE

Factor	Degrees of freedom	Mean square	F	
Variety	5	1769	68**	Error of difference between two
Locality	13	485	19**	flours = 3.6 cc
Interaction	65	26.1 <sup>1</sup>	5.3**	Based on the 5% point, difference
V × L				required for significance of:
Remainder	84	4.88		Varietal means = 2.7 cc
Total	167			Locality means = 4.2 cc

<sup>1</sup> Used as error.

\*\* Significant at the 1% point.

similar wide variations in mixograms obtained from flours produced under different environments.

*Statistical Significance of Loaf Volume Data.* The loaf volume data obtained from baking this series of flours at the natural protein level together with an analysis of variance of the data are given in Table V and for the diluted flour samples in Table VI. The analysis of variance shows that both the variety and the environment were responsible for highly significant variations in loaf volume, not only in the flours at their natural protein content but also in the flours diluted to 10% protein content. Differences required for significance were less for the diluted flour data than for the original flour data; this is to be expected since the loaves were much smaller.

Differences between locality means greater than 9.9 cc, or between variety means greater than 6.5 cc (significant differences based on the 5% point), may be considered as significant.

*Loaf Volume-Protein Relationships.* The relation of flour protein to loaf volume is shown by the regression curves on the left in Figures

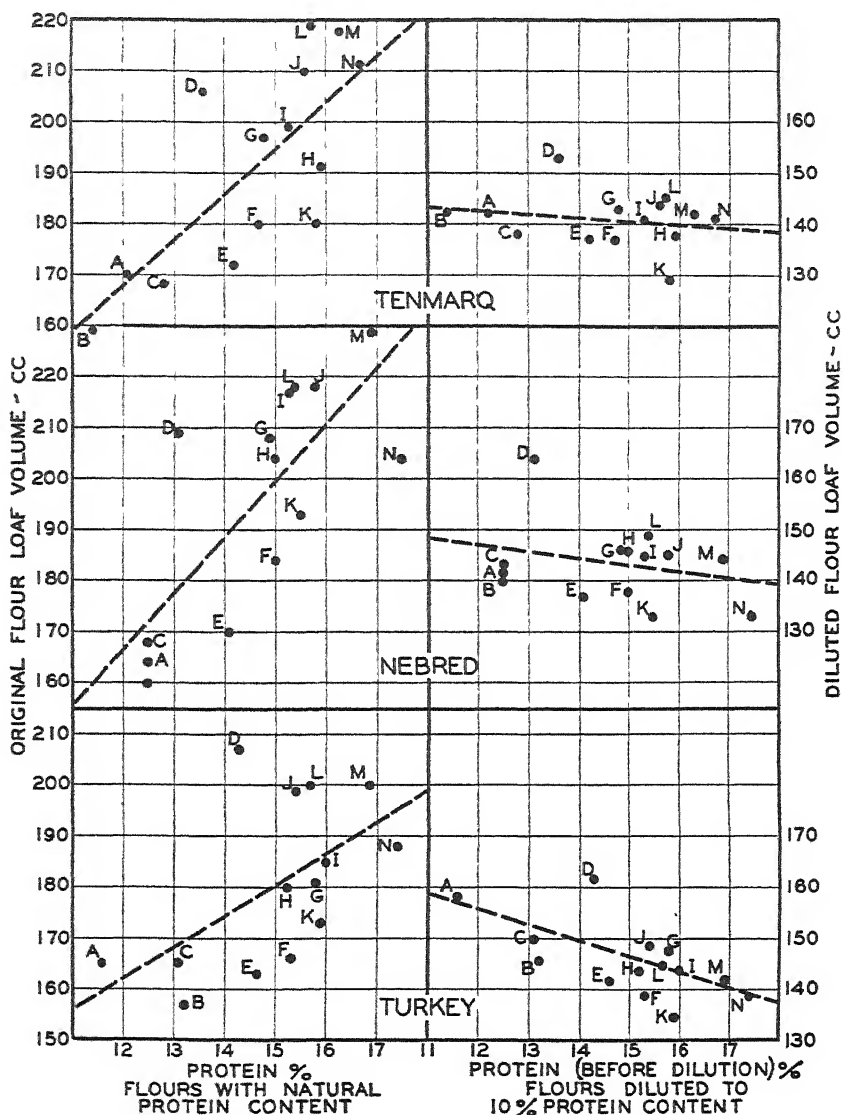


Fig. 3. Effect of variety and of environment on the relationship of loaf volume to protein. Left hand curves are for natural flours; right hand curves are for flours diluted to 10% protein content.

3 and 4. Each curve represents the results obtained from a single variety; the localities are indicated by the letters A, B, etc.

The outstanding characteristic of these curves is the deviation of

the individual samples from the calculated regression line. This is contrary to the marked lack of deviation shown by composited wheat samples (Larmour, Working, Ofelt, 1939, 1940, and McCalla, 1940). It can be seen from inspection of the curves in Figures 3 and 4 that compositing samples of nearly the same protein content or averaging the loaf volumes obtained from those having nearly equal protein (Larmour, 1931) would quite likely eliminate the variations.

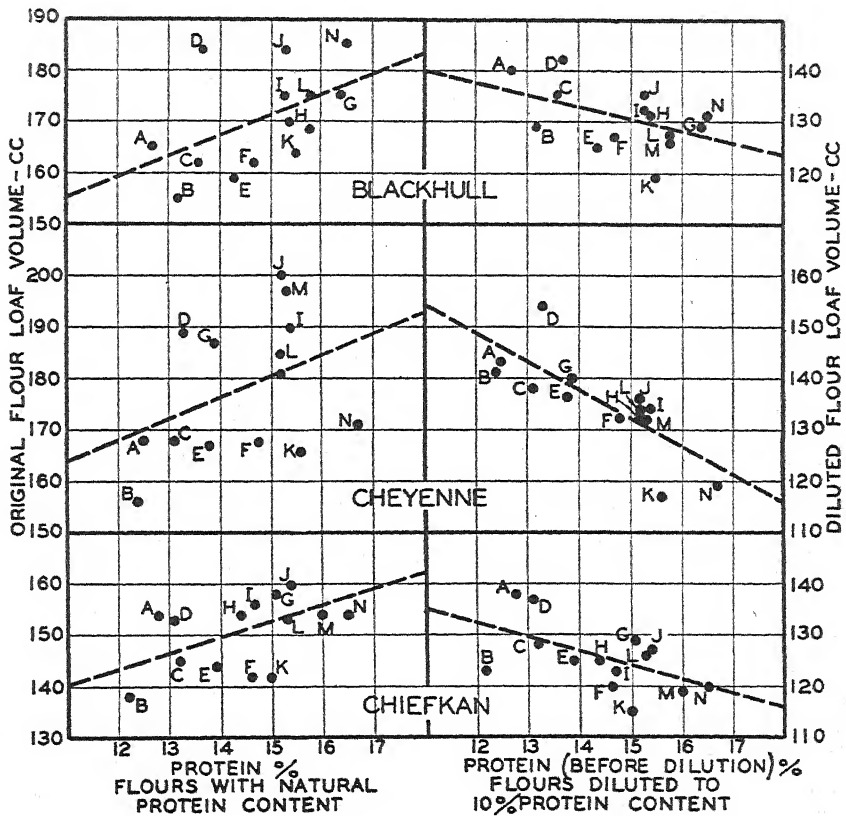


Fig. 4. Effect of variety and of environment on the relationship of loaf volume to protein. Left hand curves are for natural flours; right hand curves are for flours diluted to 10% protein content.

The marked individual variations in loaf volume were not related to protein content; *e.g.*, the environment *D* yielded flour with the capacity to produce exceptionally large loaves, whereas the environments *C* and *E* yielded flours with similar protein content but which were incapable of producing loaves with as great volume. Similarly, the environments *K* and *L* yielded wheats differing little in protein quantity but differing greatly in loaf volume capacity. Locality *D*



produced wheat with greater volume potentiality than locality *K*, even though the flours had nearly a 2% lower protein content. The quality differences were not as pronounced with Chiefkan and Black-hull wheats; nevertheless, the differences are significant and tend to parallel the other varieties at the corresponding localities.

*Effect of Varying the Baking Formula.* Such wide deviations from the expected volume-protein relationship suggest the possibility that the baking formula was not adapted to the flours from such localities as *C*, *E*, *K*, and *N*, while it might have been optimum for the flours from *D* and *L*. Accordingly, the flours from localities *C*, *D*, *L*, and *K* were rebaked, using 0.002%  $\text{KBrO}_3$  instead of 0.001% and again rebaked using 6% dry milk solids and 0.004%  $\text{KBrO}_3$  (Ofelt and Larmour, 1940).

TABLE VII  
COMPARISON OF LOAF VOLUMES OBTAINED WITH THREE BAKING FORMULAS

Variety	Locality	Flour protein	Loaf volume		
			$\text{KBrO}_3$ 1 mg %	$\text{KBrO}_3$ 2 mg %	6% D.M.S. +4 mg % $\text{KBrO}_3$
Nebred	N	%	cc	cc	cc
	F	15.4	218	213	215
	O	15.5	193	187	184
	C	13.1	209	203	212
Tenmarq	N	12.5	168	164	163
	F	15.7	219	213	210
	O	15.8	180	165	169
	C	13.6	206	200	206
		12.5	169	172	160

The loaf volumes obtained from the Nebred and Tenmarq flours using the three baking formulas are given in Table VII. It is seen that these changes in the baking formula did not materially alter the relative volumes obtained (in fact the volumes obtained with each flour by the three formulas are in surprisingly close agreement). As possible explanations for the lack of variation between these widely differing formulas, it should be noted that absorption and mixing in all cases was "optimum" and that the "rate of proof" was largely eliminated as a factor since proofing was to height. This effect of proofing to height was shown by Sandstedt and Blish (1939).

*Loaf Volume-Locality Relationships.* A comparison of the protein-volume curve for one variety with the curves of the other varieties (Figures 3 and 4) shows that, with few exceptions, the six varieties tended to respond in a similar manner to any particular set of environmental conditions; i.e., flours from certain localities are found above

the regression line regardless of variety, and flours from certain other localities are found below the regression line. However, the differences between localities are greater for certain varieties than for others. In order to show this tendency to better advantage, the Turkey wheat flour samples were arbitrarily arranged in the order of increasing loaf volumes without regard to protein content. The loaf volumes in this order were then plotted against locality; thus a fairly smooth curve for the Turkey wheat flours was obtained (Figure 5). The loaf volumes

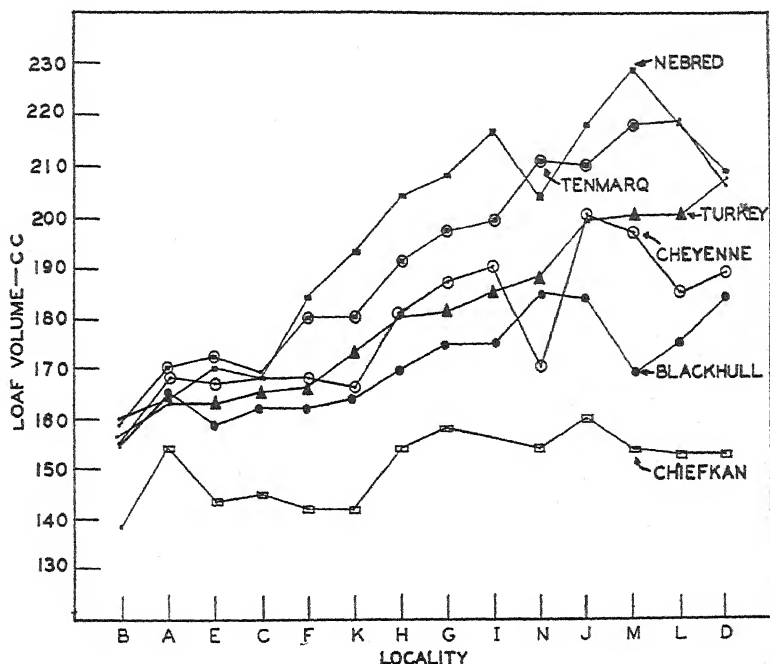


Fig. 5. Effect of locality and variety on loaf volume at the natural protein levels.

obtained from the other varieties for the respective localities were then introduced on the graph and the curves drawn for each variety. This gives a direct comparison of the loaf volumes obtained from all varieties from each locality.

This evidence gives the same general impression as was obtained from a comparison of the individual curves: the loaf volumes of the varieties had a tendency to vary in the same direction with a change in environment. The most conspicuous exceptions were the Nebred and Cheyenne samples grown under environment N.

It is quite evident that in the lower loaf volume ranges, the wheats, excepting Chiefkan, gave similar loaf volumes when grown under the

same environmental conditions, but in the higher loaf volume ranges there was a considerable spread between the varieties. The curve for Chiefkan has a tendency to remain level; *i.e.*, Chiefkan did not respond as readily as the other varieties to changes in environment. Blackhull takes a somewhat intermediate position. On the whole these data indicate that the varieties tend to respond in a similar manner to changes in environment; however, they differ markedly in the amount of response, the response being roughly proportional to the loaf volume potentiality of the variety. The interaction *variety*  $\times$  *locality*, being highly significant, also shows that the varieties did not all respond alike to the differences in environment.

*Baking Tests with Flours Diluted to 10% Protein.* The right hand curves in Figure 3 represent the loaf volumes obtained from the same flours as shown in the left hand curves after they had been diluted with starch to an artificial 10% protein content. This dilution was used to eliminate quantity of protein as a variable not only in estimating quality from loaf volume but also as a variable in evaluating such other characteristics as grain, texture, and handling properties. It is exceedingly difficult to evaluate these factors in flours of unequal protein content since "buckiness" and a tendency toward extreme open grain are characteristic of high-protein strong flours, especially when supplements are used in the baking test to obtain maximum volumes. The 10% protein level was chosen so that the lower protein wheats could be included in this comparison. Generally, there might be considerable advantage in diluting to a protein level similar to that used in average commercial baking.

The loaf volume curves from the diluted flours indicate much the same variation in quality in any one wheat variety as was shown by the data for the original flours. There is an evident similarity between the distribution of the natural and the diluted flours in respect to their regression lines. A comparison of the relation of the individual samples of natural and diluted flours to their respective regression lines indicates that much the same information may be obtained about the individual samples by either baking procedure; the exceedingly high quality of the wheats grown under environment *D* and the poor quality of those grown under environment *K* is apparent.

*Relation of Quality to Protein Content.* The 14 samples of flour representing each variety are too few to give a dependable varietal regression line; nevertheless, these regression lines for the diluted flours seem to indicate that in the varieties having the highest loaf volume potentialities (Nebred and Tenmarq), quality had a tendency to be independent of protein content (the regression coefficients, though negative, were small), while in the other varieties quality tended to

decrease with an increase in protein content. The regression curves obtained by Sandstedt and Ofelt (1940) suggested a similar relationship; however, their data also indicated lower quality in samples having low protein content. The flours included in the present report were not of sufficiently low protein content to check this possibility.

*Blending Values.* It was suggested by Sandstedt and Ofelt (1940) that the baking results obtained by diluting with starch to a definite protein level might be an indication of the efficiency of the protein of the flours for blending purposes. On this basis, the proteins of Turkey, Nebred, and Tenmarq grown under this particular set of conditions were about equal in blending value and somewhat better than the other varieties. Differences in blending efficiency between varieties were greater in the higher protein ranges. If the loaf volumes obtained from these diluted flours are taken as an index of their blending efficiency, these results indicate that blending efficiency is not only a varietal characteristic but is to a large extent dependent on environment and in some varieties also on protein content. Chiefkan, as a variety, was low in volume; however, Chiefkan grown at *A* and at *D*, diluted to 10% protein, gave loaf volumes as large as, or larger than, Blackhull grown at the other localities and as large as Tenmarq and Nebred grown at *E*, *C*, *F*, and *K*.

### Summary

Baking properties of hard winter wheats were found to be markedly affected by the environments in which the wheats were grown. All varieties from one locality showed high absorptions; from another locality, poor handling properties; from others, shorter or longer than normal mixing requirements; and from others, exceptional loaf volume potentialities. These variations from the expected behavior were largely independent of protein content.

Quality in some varieties seemed to be practically independent of protein content, but in other varieties quality decreased with increase in protein content.

Though the varieties tended to respond in a similar manner to environmental changes, the degree of response was determined by variety. Varieties with low loaf volume potentiality gave the least loaf volume response to changes in environment, while varieties with high loaf volume potentiality gave the greatest responses; similarly, varieties with the shortest mixing requirement gave the least response in mixing requirement to environmental change, while varieties with the longest mixing requirement gave the greatest response. Correspondingly, the differences in loaf volume between varieties were greatest in localities which produced the flours with the largest loaf

volume potentialities and the differences in mixing requirement between varieties were greatest in localities producing the longest mixing requirements.

Blending efficiency of the flour protein, while largely dependent on variety, may be materially affected by environment and in some varieties may decrease with increase in protein content.

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## A MICROSCOPIC STUDY OF THE BEHAVIOR OF FATS IN CAKE BATTERS

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(Presented at the Annual Meeting, May 1943; received for publication May 19, 1943)

The basic structure of cake batters has long been a matter of speculation. The fat-air dispersion phenomenon, not to mention the fat-liquid emulsification state, has proved an intriguing subject to those interested in the fundamentals of cake baking. The common use of emulsifying agents in cake shortenings has increased the interest during the past few years. Because of the subject's complexity, much of the information published to date deals with theoretical considerations. Morris (1929) concluded that the air cells present in cake batter are surrounded by a film of sugar syrup and egg protein, or both, which in turn is emulsified in fat. Grewe (1937) reported that creamed mixtures of sugar, fat, and eggs represent a water-in-oil emulsion. On the bases of viscosity and electrical conductivity studies, Sunderlin and Collins (1940) concluded that thin batters are oil-in-water emulsions whereas thick batters are water-in-oil emulsions. Their data indicate that there is a gradual transition from one type of emulsion to the other. Morr (1939) made microscopical examinations of baked cakes and showed that the starch and fat of cakes are imbedded in or at the surface of the protein matrix. It was indicated that hydrogenated fats collect in clumps of fairly large size at the cake-air interface, that butter appears to be more finely divided than hydrogenated fats and appears to be distributed throughout the entire crumb, and that liquid fats seem to collect in pools at the cake-air interface with only a small portion distributed within the crumb. Lowe (1943) indicated that thin batters produced inferior cakes whereas viscous ones produced more desirable cakes. Lowe speculated, "If in the thin batters, the fat is largely dispersed as an oil-in-water emulsion and in the viscous ones as a water-in-oil emulsion, it is not impossible to have both types of emulsion in the intermediate batters. Not all of the fat may be emulsified." Lowe finally concluded that fats may possibly be dispersed in cake in several ways, such as an oil-in-water emulsion; a water-in-oil emulsion; films, pools, or lakes throughout the cake ingredients or adsorbed on the starch and protein of the crumb as monomolecular (or possibly multimolecular) films; at the cake-air interface; or by a combination of all or some of these.

Bailey and LeClerc (1935) supported the theory that a water-in-fat emulsion is formed during the creaming of cake batter.

Based on a microscopic examination of cake batter, Sunderlin and Collins (1940) concluded that gas bubbles were present in batter in grapelike clusters.

The work reported in this paper was undertaken to develop additional information regarding the basic structure of cake batters containing fats; to study the effect of emulsifying agents on cake batter dispersion; to determine the fate of suspended air cells during the baking process; to observe the structural changes taking place during baking; and to attempt to correlate known cake defects such as shrinkage and texture irregularities to some controllable factor or factors.

### Materials and Methods

Cake types were both pound cake and white layer cake. In the case of pound cake, the percentage of fat was relatively high when compared with the white layer cake formula. The batter temperature was maintained at 75°F in all cases, unless otherwise stated.

After the batter was mixed, a minute portion was transferred to a glass slide and a cover glass was pressed down until the batter was extended sufficiently to become transparent to reflected light.

The fat was colored with fat soluble dyes, either yellow AB<sup>1</sup> or OB,<sup>2</sup> and consequently it became relatively easy to follow the behavior of the fat prior to and during baking. Observations were recorded by means of photomicrographs. Higher magnifications (up to 225 X) were of value in observing the behavior of single air spaces and single fat areas, while lower magnifications (25 X) were necessary for the study of several fat areas concurrently.

To permit a study of the changes which occur in the structure of the batters during baking, the microscope was specially equipped with a heating stage. The stage consisted of a resistance coil covered by a steel plate. The coil was operated from a 6-volt transformer and its temperature could be controlled, within limits, by means of a rheostat. Batters which were cooked or baked on slides by means of the heating stage were compared with similar batters taken from partially baked batter removed from cake layers during the regular process of baking.

The point at which the batters became "baked" was determined by means of polarized light. Upon the completion of the baking process, the characteristic birefringence of the starch granule disappeared. This loss of birefringence when a film of the batter was baked on the slide was confirmed with cakes baked in a normal manner.

<sup>1</sup> Benzeneazo naphthylamine—a certified dye used as a butter color.

<sup>2</sup> O-toluenazo naphthylamine, also used as a butter color.

## Results and Discussion

*The Effect of Emulsifying Agents on Fat Dispersion.* Both pound cake batters and white layer cake batters were made with hydrogenated shortening and with similar shortening containing varying percentages of mono- and di-glyceride emulsifying agent. Macroscopically the batters appeared "curdled" without the presence of emulsifying agent. Added quantities of emulsifying agent (up to 10% of the total shortening) overcame this curdled appearance and developed a thin but smooth batter. The viscosity of the batter decreased with each added quantity of emulsifying agent; the batter specific gravity increased, the cake volume increased, and the fat lake areas became more finely dispersed.

The findings of Grewe (1937) were verified. By means of electrical conductivity tests, it was proved that a water-in-fat emulsion existed during the creaming of fat, sugar, and eggs. It is difficult to attach much significance to the emulsion state at this particular stage of the cake making process. Just as soon as flour was added to this creamed mass the conductivity of the mixture increased almost to that of the emulsified aqueous medium. Apparently the major portion of the liquid (with its dissolved electrolytes) was released from its emulsified state by the addition of flour. It was evident that a continuous water-in-fat emulsion did not exist in the batters studied. Of course this does not eliminate the possibility of combination emulsions; *i.e.*, water-in-fat clumps distributed through an aqueous-flour medium.

*Cake Batter at Varying Magnifications.* The photomicrograph in the upper left corner of Figure 1 shows the structure of white layer cake batter as observed at 50 magnifications. The dark, irregular clumps or "lakes" are composed of fat, while the continuous field is made up of the aqueous phase with its dissolved sugar, salt, and baking powder, and its suspended flour and eggs. It will be noted that the air spaces are suspended only in the fat lakes.

The upper right photomicrograph is similar to that of the upper left corner, except that the magnification is greater. The air cells with their surrounding coating of fat are more clearly depicted. The flour particles suspended in the aqueous medium can be easily observed. No undissolved sugar and salt can be seen.

The lower left and right photomicrographs are also at 50 and 225 magnifications respectively. The flour particles have been stained by the addition of an iodine solution to more clearly depict their locations.

It is obvious from a study of the photomicrographs of Figure 1 that the fat is distributed through the batter in the form of small clumps or lakelike areas. The clumps of fat hold in suspension all of



the air which the batter contains. There is a complete absence of air spaces in the aqueous-flour areas. This is an important point and represents one about which there is considerable discord. It is also apparent that the fat does not represent the continuous phase of the emulsion.

Figure 2 shows a photomicrograph of white layer cake batter containing regular hydrogenated shortening without added emulsifying

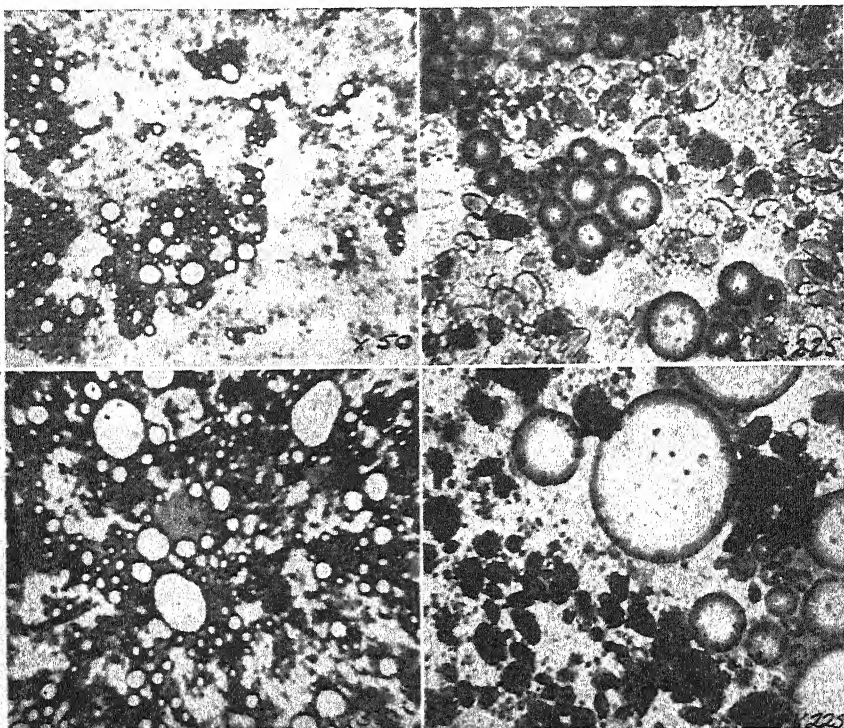


Fig. 1. Photomicrographs of white layer cake batter at varying magnifications. Upper left and right: 50 and 225 magnifications respectively. Lower left and right: 50 and 225 magnifications respectively (iodine added).

agent. The fat is distributed in the form of fat lakes or clumps and air spaces are present in each fat lake. Cakes baked from this batter shrank excessively upon baking, indicating a highly unstable cellular structure.

The shortening employed in preparing the batter for Figure 3 (upper) was the same as that for the batter of Figure 2, with the exception that 0.1% of a monoglyceride emulsifying agent was dissolved in the shortening. This resulted in the formation of more fat

lakes per unit area than were present in the batter which contained no added emulsifying agent. For the lower photomicrograph of Figure 3, the percentage of added emulsifying agent was increased to 0.25%. It can be readily seen that the additional emulsifying agent has resulted in a much larger number of fat lakes per unit area of batter.

The three photomicrographs at the left in Figure 4 indicate the progressive increase in the number of fat lakes per unit area with increases of emulsifying agent to 0.5%, 1%, and 3% respectively. Each increase in the quantity of emulsifying agent reduced cake shrinkage and resulted in cakes of generally superior quality.

The photomicrographs on the right in Figure 4 indicate the decreasing size of each fat clump with each increase in monoglyceride emulsifying agent. It is still evident that all air bubbles are sur-

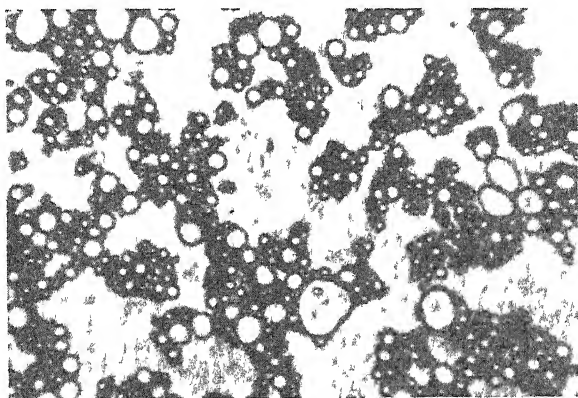


Fig 2 Photomicrograph of white layer cake batter containing hydrogenated shortening (50 X).

rounded by fat and that few, if any, have entered the aqueous medium free from a protective fat coating.

With each addition of emulsifying agent, up to the level of 5 to 6%, there was a corresponding increase in cake volume. After the 5% level was reached, the cake volume approached a maximum, and at 8 and 9% the cake volume began to decline, indicating a detrimental effect when fat is dispersed in too fine a pattern.

The specific gravities of the batters became greater as the quantity of emulsifying agent was increased. There was a perceptible increase in specific gravity between 0 and 1%, with a gradual flattening out of the curve at the higher percentages. Batter viscosity was also checked and it was found that the batters containing emulsifying agent were less viscous than those made without.

The effect of increasing percentages of monoglycerides on the number of fat lakes per unit area and on batter viscosity is shown in Figure 5. The rate of change in viscosity is roughly inversely proportional to the rate of change in the number of fat lakes per unit area.

From this study sufficient data are not available to imply that there is a correlation between viscosity and the number of fat lakes

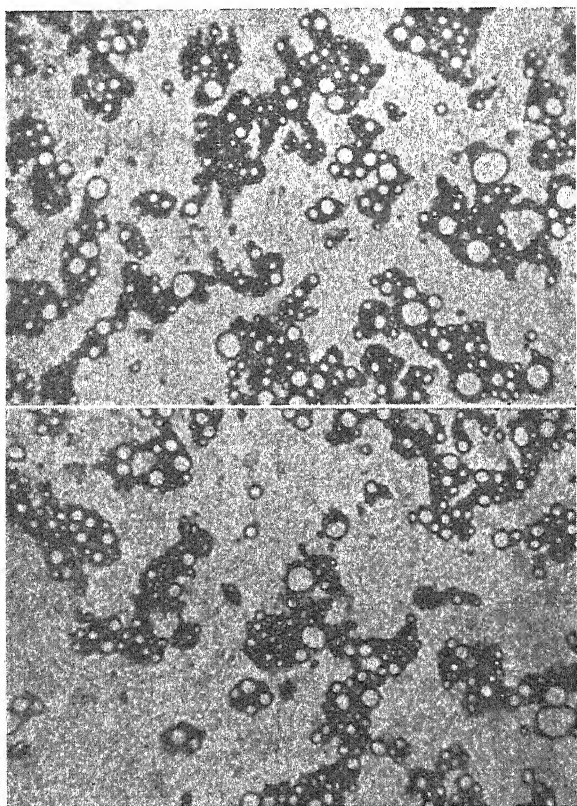


Fig. 3. Photomicrographs of white layer cake batter (50 X). Upper—0.1% monoglyceride emulsifying agent added. Lower—0.25% monoglyceride emulsifying agent added.

distributed through the batter; however, this appears to be an important factor. Since there is considerable discordant literature concerning the harmful effect of low viscosity batters on cake volume and other cake characteristics, it is only pertinent to point out that, within limits, the reverse of this contention is indicated, particularly if batters of lower viscosity are obtained by means of a finer fat dispersion.

*Behavior of Lard in Cakes.* Through photomicrographic studies it was indicated that lard disperses throughout cake batter in a much

finer pattern than that of hydrogenated vegetable shortenings. In fact, its dispersal pattern is somewhat similar to that obtained with hydrogenated fats containing emulsifying agents. There is one im-

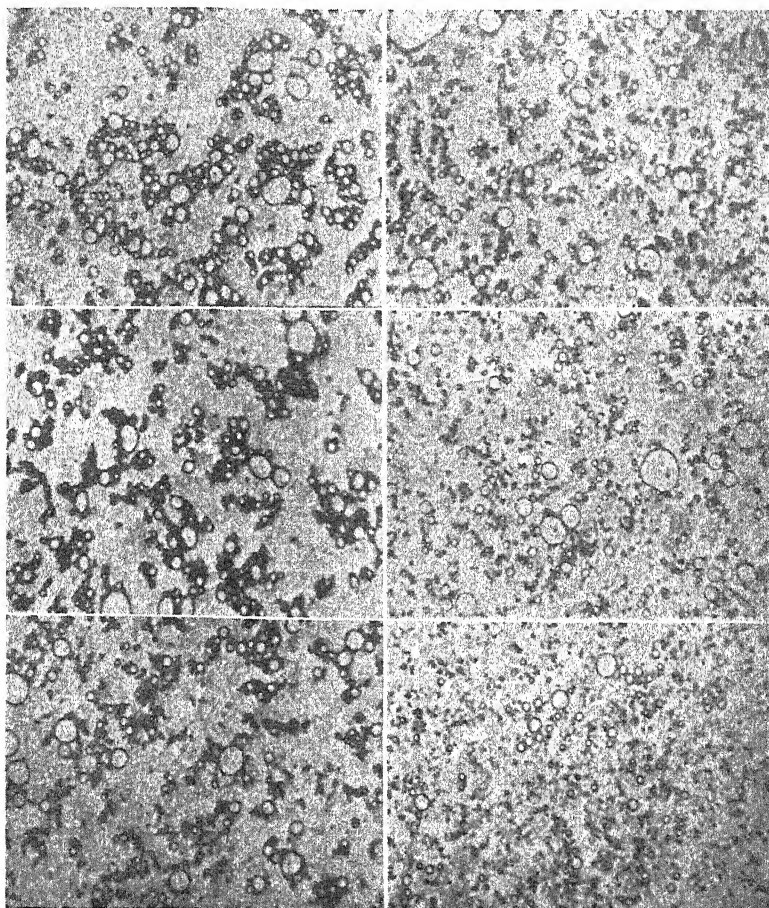


Fig. 4. Photomicrographs of white layer cake batter (50 X) showing the effect of increasing quantities of emulsifying agent in fat dispersion. Percentages given below refer to monoglyceride added.

Left upper..... 0.5%  
Left middle..... 1.0%  
Left lower..... 3.0%

Right upper..... 5.0%  
Right middle..... 7.0%  
Right lower..... 9.0%

portant difference: the quantity of air which is suspended in the fat lakes is reduced when lard is used as the shortening agent. This fine dispersion pattern explains why lard gives much better results in the richer type (140% sugar) layer cakes than does regular hydrogenated

shortening which contains no added emulsifying agent. Lard gives a fairly satisfactory performance in cakes of this type while hydrogenated shortenings without emulsifying agents produce failures. If the dispersal pattern normal to lard is changed through alteration of either its composition or texture to the point where the fat lakes in the batter become larger, the behavior of lard then approximates that of hydrogenated shortenings, and the ability to produce cakes of high sugar and moisture content without resorting to the use of emulsifying agents is thereby lessened. Conversely, the ability of lard to suspend air cells is increased when the fat dispersal pattern becomes coarse. Both air suspension and fat dispersal properties of lard can be regulated at any desired level.

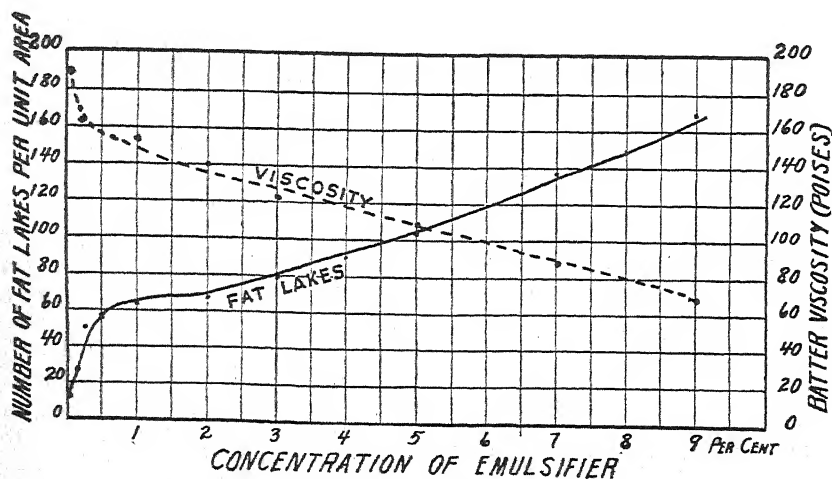


Fig. 5. Effect of concentration of monoglycerides present in shortening on the number of fat lakes and the viscosity of white layer cake batters.

*The Behavior of Baking Powder in Cakes.* A photomicrographic study of layer cake batters containing baking powder indicates that very few, if any, new gas cells are formed as a result of the baking powder reaction. The released carbon dioxide gas seems to collect at the air space interface, and each existing space seems to grow larger. It is significant that new air spaces are not formed. This observation further emphasizes the role played by creamed-in air cells in the expansion of cake batters during baking.

*Observations of Batter Structure During Baking.* Photomicrographs of batter made during the baking process are shown in Figure 6. After 5 min of baking had elapsed, it was found that the fat had melted and had released the air cells held in suspension. These cells were thereby

transferred from the fat phase to the aqueous medium. The fat, freed of its air bubble structure, collected in small lakes throughout the baking batter. Obviously these fat globules were smaller and in greater number when emulsifying agents were used. It is

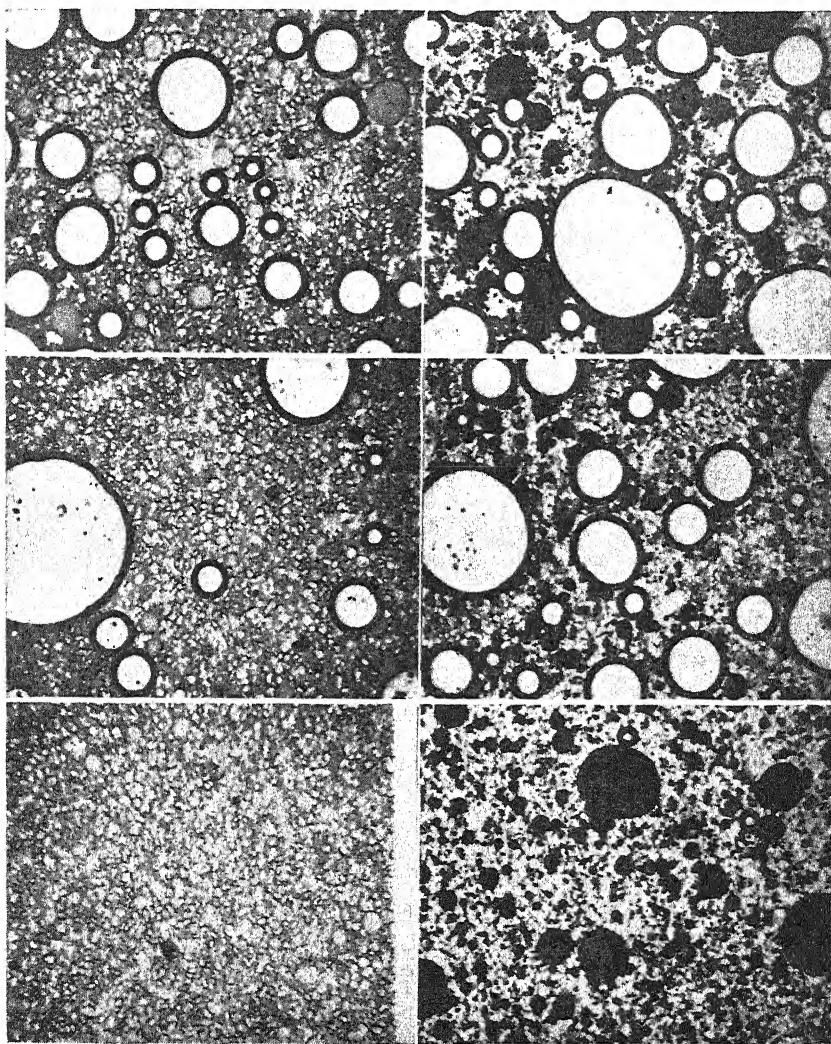


Fig. 6. Photomicrographs (100 X) of white layer cake batters made with and without emulsifying agent at various stages of the baking process. Upper, middle, and lower pairs represent 5-min, 10-min, and 15-min intervals respectively. In each pair, the picture at the left shows batter containing emulsifying agent; at right, without emulsifying agent. At the 5-min interval the batter was just beginning to expand; at the 10-min interval the batter had attained a high degree of expansion. After baking for 15 min it was impossible to transfer samples to the slide without loss of air; accordingly the photomicrographs for this time interval do not represent the actual batter conditions.



popularly believed that a greater distribution of fat, brought about by the use of emulsifying materials, will cause increased tenderness. Our work indicated that the reverse is true, because the cakes which contained no added emulsifying agents were far more tender than those containing the emulsifier.

As the batter continued baking, a movement of the air spaces was observed. The movement appeared to flow with the aqueous medium along the path of a convection current. The fat globules moved along the same path. There was little coalescence between separate fat areas. On the other hand, air spaces coalesced quite readily, as the larger cells often absorbed the smaller ones. As the end of the baking process was neared, a sudden increase in internal pressure was observed. This was reflected by rapid movement and distortions of the air spaces. Air spaces appeared to explode. The fat globules seemed to present points of extreme mobility. The movement during the period of high pressure was so vigorous that cake tunnels, holes, and other texture irregularities may be formed under certain conditions. It is even more significant that the phenomenon of shrinkage (which is partly overcome by the use of emulsifying agents) occurs at this stage of the baking process. The size of the fat globule possibly affects the stability of the cake structure and thereby influences cake shrinkage.

### Summary and Conclusions

Layer and pound cake batters appear to be suspensions of air bubbles in fat distributed in a medium of flour and liquid. Little, if any, liquid appears to be emulsified in the fat. Soluble ingredients such as salt and sugar are dissolved by the water of the batter. The air spaces in layer and pound cake batters are invariably surrounded by fat.

During baking, the fat quickly melts and releases its suspended air to the flour-water medium. Gas produced by baking powder finds its way into the air spaces already existing within the batter. The completion of the baking process may be determined microscopically by means of polarized light. The cross pattern of wheat starch disappears at this stage.

There is a movement of air spaces at all times during the baking process. This movement appears to follow a definite convection pattern until the end of the baking process is neared. At this stage the movement becomes violent and without direction.

The use of monoglyceride type emulsifying agents produces a finer dispersion of fat throughout the cake batter.

## Acknowledgment

The writer wishes to express appreciation to Mr. Harry Brody for his diligence in the preparation of numerous batches of cakes required by this study. In addition, acknowledgment is expressed to Drs. Lyle St. Amant and C. H. Koonz of the Histological Division for the vast amount of effort expended in the preparation of the photomicrographs. Without the cooperative effort of these individuals, this study would not have been possible.

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EFFECT OF SCAB ON THE QUALITY OF HARD RED SPRING WHEAT<sup>1</sup>

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(Received for publication November 26, 1943)

Scab infections, caused chiefly by the fungus *Gibberella zeae* (Schw.) Petch (*G. saubinetii* Auct.), have resulted in substantial damage to hard red spring wheat in certain sections of Minnesota in recent years (Christensen and Rose, 1941). This damage has occurred chiefly in the south and west central sections of the state, but not to any appreciable extent in the northwestern part where a large portion of the spring wheat is grown. In 1941 and 1942 scab was a serious problem in the south central region, and in 1942 the infection spread to the west central part. In the latter year there was an average infection of 13% in the varieties of spring wheat grown in the one-fortieth acre plots at the Southeast Experiment Station at Waseca, and at the West Central Station at Morris. Four varieties of hard red spring wheat grown at the latter station for milling and baking tests for the Northwest Crop Improvement Association were so badly damaged with scab that they

<sup>1</sup> Cooperative investigations of the Minnesota Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture. Paper No. 2119 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

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were considered to be unfit for comparative quality studies with varieties grown in other sections.

As a substantial quantity of scabby wheat is marketed, with little published information available regarding the effects of various proportions of scab-damaged wheat on physical and chemical characteristics or on milling and baking quality, a study was undertaken. The results are described in this paper.

### Materials and Methods

Four hard red spring wheat varieties were included in this study: Thatcher (C.I. 10003),<sup>3</sup> Regent (C.I. 12070), Ceres—Double Cross × Ceres-Hope-Florence (Ns 2829,<sup>3</sup> C.I. 12008), and Mercury<sup>2</sup> × Comet-1018 (Ns 2822, C.I. 12071). The prevalence of scab was estimated in the standing grain before harvest and in the threshed grain. The former was derived by first estimating the average percentage of infected heads and multiplying this by the estimated percentage of infected florets in these infected heads. The infections for the four varieties so determined were 70, 60, 60, and 80%, respectively. Infection in the threshed grain was determined by examining each kernel in a sample of each variety, those being considered as scabby which had the characteristic grayish color. The percentages of infected kernels were found to be 16, 16, 12, and 20. These lower percentages are thought to be due to failure of many of the infected florets to produce grain and to removal of badly infected light-weight kernels by the fan and screen of the ordinary farm thresher.

The samples were divided into two lots designated as "sound" and "scabby." These were prepared by first blowing out as many of the light scabby kernels as possible with a blower, and then removing the remaining visibly damaged kernels by hand picking. The scabby wheat thus removed was composited to form the scabby sample. The sound samples contained no more than 1% of scabby grain and the scabby samples were at least 95% scabby.

The internal microflora of the sound and scabby samples was studied by means of a surface-sterilization technique followed by plating the wet kernels on sterile, acidified nutrient agar. Approximately 100 seeds were dipped in 70% ethanol, followed by immersion in mercuric chloride solution (1 : 1,000) for about 2 min. To minimize contamination with air-borne organisms, the kernels were then rinsed with dilute calcium hypochlorite solution and transferred to nutrient agar plates. Readings of the number and types of organisms arising from the seed were made after incubation for 7 days at room temperature. This technique is primarily designed for the determination of

<sup>2</sup> "C.I." refers to accession number of the Division of Cereal Crops and Diseases, U.S.D.A.; "Ns" refers to North Dakota number.

fungi, but certain bacteria also grow under these conditions; bacteria and fungi other than *Gibberella*, *Helminthosporium* and *Alternaria* were not classified.

Determinations of test weight per bushel, moisture, protein, and ash were made on the wheats by the procedures given in Cereal Laboratory Methods (4th ed., 1941). Weight per 1,000 kernels was obtained. The apparent specific gravity of the wheats was determined by measuring, in a 25 ml burette, the volume of an ethanol-carbon tetrachloride mixture (1 + 1) displaced by a 5-g sample.

After scouring, the wheats were milled at a moisture content of 16.2% in a six-stand experimental mill. The weight of total flour recovered was employed in calculating flour yield; the patent flour (about 85% patent) was reserved for analytical and baking tests.

The patent flours were analyzed for moisture, protein, ash, total and reducing sugars, and carotinoid pigment content by the procedures given in Cereal Laboratory Methods (4th ed., 1941).

Experimental baking tests were made with the A.A.C.C. formula, using a National dough sheeter, low-form pans, and doughs scaled to 150 g. The flours milled from sound and scabby wheat for two of the varieties, Thatcher and Ns 2829, were baked in duplicate, employing combinations of the bromate levels (0, 1, and 3 mg potassium bromate per 100 g of flour), three mixing times (1, 2, and 4 min in a Hobart-Swanson mixer), and two fermentation periods (2 and 3 hr). Since in commercial practice scabby wheat would be milled in blends with sound grain, it seemed desirable to carry out baking tests on blends of the flours milled from sound and scabby wheat of the same variety. For each variety, blends containing 10 and 30% of flours milled from the scabby wheat were mixed with the corresponding flour from the sound wheat. These blends were baked in duplicate by the A.A.C.C. formula with the addition of 1 mg potassium bromate. The doughs were mixed for 2 min and fermented for two periods (2 and 3 hr).

As a measure of the effect of scab infection on the changes in dough consistency during fermentation, farinograph curves were made with doughs prepared according to the regular baking formula and containing 1 mg potassium bromate. These tests were confined to flours milled from the scabby and sound wheats obtained from Thatcher and Ns 2829. The doughs were mixed to minimum mobility, allowed to ferment in the farinograph for one hour, and then remixed for 10 min; after an additional hour of fermentation, the doughs were again remixed.

### Results and Discussion

The internal microflora of the samples are given in Table I. Classification of the original samples into "sound" and "scabby" fractions

TABLE I  
INTERNAL AEROBIC MICROFLORA OF SOUND AND SCABBY WHEATS

Variety and condition		Clean seed	Seeds infected with fungi and bacteria <sup>1</sup>				
			<i>Gibberella</i>	<i>Helminthosporium</i> sp.	<i>Alternaria</i> sp.	<i>Gibberella</i> , <i>Helminthosporium</i> and/or <i>Alternaria</i> <sup>2</sup>	Miscellaneous fungi + bacteria
Regent	sound	2	1	45	54	9	7
	blighted	7	33	31	30	7	4
Thatcher	sound	30	0	19	46	1	5
	blighted	21	30	8	34	3	6
Ns 2822	sound	27	6	8	55	0	4
	blighted	18	38	4	37	2	3
Ns 2829	sound	40	3	10	43	0	4
	blighted	6	27	7	63	6	3
Mean	sound	25	2	20	50	2	5
	blighted	13	42	12	41	4	4

<sup>1</sup> The total percentages sometimes exceed 100% because some kernels yielded two kinds of fungi.

<sup>2</sup> The values in this column represent the percentages of seeds infected with two or more species.

on the basis of kernel color was quite satisfactory in separating the kernels which were diseased with *Gibberella*. Both classes of samples were, however, appreciably infected with other microorganisms, particularly *Helminthosporium* sp. and *Alternaria* sp. Since the extent of infection with microorganisms other than *Gibberella* is about the same in both series of samples, differences in chemical composition and in milling and baking properties may be attributed, in large part, to infection with *Gibberella*.

The test weight, weight per 1,000 kernels, flour yield, apparent specific gravity, and ratio of flour yield to apparent specific gravity are given in Table II.

TABLE II  
PHYSICAL PROPERTIES AND FLOUR YIELD OF SOUND AND SCABBY WHEAT

Variety and condition		Test weight	Weight per 1,000 kernels	Flour yield	Specific gravity	Flour yield / Specific gravity
		lb/bu	g	%		
Thatcher	sound	61	20.70	74.2	1.402	52.9
	scabby	56	16.30	73.1	1.378	53.0
Ns 2829	sound	62	36.24	76.2	1.381	55.2
	scabby	57	26.67	75.0	1.362	55.1
Regent	sound	61	31.48	78.1	1.389	56.2
	scabby	56	24.76	76.1	1.351	56.3
Ns 2822	sound	61	33.45	75.3	1.381	54.5
	scabby	52	22.59	73.5	1.346	54.6
Mean	sound	61.2	30.47	76.0	1.388	54.7
	scabby	55.2	22.58	74.4	1.359	54.8

The test weight of the scabby wheat averaged 6 lb less than that of the sound wheat. This difference would have been greater but for the fact that the scabby samples contained some kernels which were only slightly shrivelled but which were affected by scab, as shown by a gray color. Moreover, many of the very light, scabby kernels were lost during the threshing and cleaning operations; this would also occur in commercial practice. The variety Ns 2822 was most affected by scab and showed the greatest difference between the test weight of the sound and scabby wheat.

The weight per 1,000 kernels averaged 7.9 g lower for the scabby than for the sound wheat. Thatcher had the lowest and Ns 2829 the highest values; these varietal differences are a direct reflection of variations in the average size of the kernels.

The flour yield was slightly but consistently less for the scabby than for the sound wheat. It averaged 1.6% less. This is a smaller difference than would be expected from the lower test weight of the scabby wheat. It is, however, highly significant, statistically, as shown by a variance analysis of the data. The scabby wheat presented no particular difficulties in milling.

As expected, the specific gravity was lower for the scabby than for the sound grain. The ratios of flour yield to specific gravity were the same for sound and scabby wheat for any given variety but differed between varieties.

The chemical analyses of the wheats and patent flours are recorded in Table III. No consistent differences were found between the pro-

TABLE III  
EFFECT OF SCAB INFECTION ON CHEMICAL COMPOSITION

Variety and condition		Wheat <sup>1</sup>		Patent flour <sup>1</sup>					
		Protein	Ash	Protein	Ash	Reducing sugar	Total sugar	Non-reducing sugar <sup>2</sup>	Carotinoid pigment <sup>3</sup>
		%	%	%	%	%	%	%	ppm
Thatcher	sound	13.0	1.52	12.3	0.44	0.17	1.05	0.88	2.64
	scabby	13.3	1.62	12.5	0.50	0.23	1.13	0.90	3.18
Ns 2829	sound	15.2	1.72	14.1	0.42	0.22	1.14	0.92	1.91
	scabby	14.8	1.82	13.6	0.53	0.34	1.15	0.81	2.51
Regent	sound	14.1	1.48	13.7	0.41	0.23	1.34	1.11	2.44
	scabby	14.3	1.54	13.8	0.50	0.32	1.32	1.00	2.88
Ns 2822	sound	14.9	1.42	14.4	0.40	0.20	1.03	0.84	2.52
	scabby	14.2	1.57	13.4	0.51	0.31	1.18	0.87	3.18
Mean	sound	14.3	1.54	13.6	0.42	0.20	1.14	0.94	2.38
	scabby	14.2	1.64	13.3	0.51	0.30	1.20	0.90	2.94

<sup>1</sup> Analyses are expressed on a 15% moisture basis.

<sup>2</sup> Difference between total and reducing sugars.

<sup>3</sup> Expressed as carotene.

TABLE IV

MEAN LOAF VOLUMES OBTAINED IN DIFFERENTIAL BAKING TESTS WITH FLOURS  
MILLED FROM SOUND AND SCAB INFECTED WHEAT

Mix- ing time	Potassium bromate	Variety					
		Thatcher		Ns 2829		Both varieties	
		Sound	Scabby	Sound	Scabby	Sound	Scabby
<i>min</i>	<i>mg</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>

## FERMENTATION TIME—2 HR

1	0	673	693	755	682	714	688
	1	688	710	820	748	754	729
	3	675	745	943	793	809	769
	Mean	679	716	839	741	759	728
2	0	710	715	798	705	754	710
	1	780	810	915	808	848	809
	3	843	815	810	808	826	812
	Mean	778	780	841	774	810	777
4	0	795	798	788	695	792	746
	1	895	898	858	733	876	816
	3	808	830	790	735	799	782
	Mean	833	842	812	721	822	782
	Mean (2 hr)	763	779	831	745	797	762

## FERMENTATION TIME—3 HR

1	0	658	675	765	730	712	702
	1	710	723	860	800	785	762
	3	653	658	825	743	739	700
	Mean	675	685	817	758	746	722
2	0	660	713	770	735	715	724
	1	783	760	883	813	833	786
	3	698	710	813	768	756	739
	Mean	714	728	822	772	768	750
4	0	720	710	765	670	742	690
	1	770	795	790	703	780	749
	3	603	625	660	660	632	642
	Mean	699	710	738	678	718	694
	Mean (3 hr)	695	708	792	736	744	722
	General mean	729	744	812	740	770	742

TABLE IV—(Continued)  
ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	F
<i>Flours</i>	3	60,208	50.1†
Variety, V	1	74,029	61.6†
Scab, S	1	40,501	39.7†
V × S	1	66,093	55.0†
<i>Treatments</i>	17	23,098	19.2†
Fermentation period, F	1	79,101	65.9†
Mixing time, M	2	22,423	18.7†
Bromate level, B	2	58,349	48.6†
F × M	2	17,599	14.7†
F × B	2	32,192	26.8†
M × B	4	12,238	10.2†
F × M × B	4	872	—
<i>Flours × treatments</i>	51	3,517	2.9*
V × F	1	11,290	9.4†
V × M	2	49,918	41.6†
V × B	2	5,778	4.8*
S × F	1	5,078	4.2*
S × M	2	568	—
S × B	2	617	—
Remainder	41	1,201	—
Between duplicates	72	691	—
Total	143		

\* Exceeds 5% point by comparison with "remainder" mean square.

† Exceeds 1% point by comparison with "remainder" mean square.

tein content of the sound and scabby wheats, but the latter were slightly higher in ash content. The flours milled from the scabby samples were slightly but consistently higher in ash and reducing sugar content, and were considerably higher in carotinoid pigment content than the corresponding flours from the sound wheats.

The mean loaf volumes for the differential baking tests, conducted with flours milled from the sound and scabby wheats obtained from Thatcher and Ns 2829, are recorded in Table IV, together with a variance analysis of the data.

For both varieties combined, the mean loaf volume for all baking treatments of the flours milled from the scabby wheats was significantly lower by 28 cc than the corresponding mean for the sound wheat. However, the average loaf volume from the scabby wheat of Thatcher was 15 cc greater than from the sound wheat, while the average volume from the sound grain of Ns 2829 was 72 cc greater than from the scabby grain. The loaf volumes of the flours milled from the scabby and sound wheat were similarly influenced by variations in mixing time and bromate level and showed only slight differences in regard to the effect of varying fermentation times. These data indicate that while

scab infection may sometimes result in flours of lower strength, as measured by loaf volume, the mixing, fermentation, and oxidation requirements are not appreciably influenced.

Aside from loaf volume, it is of importance to consider the effect of scab infection on other baking characteristics. Flours from sound and scabby wheats required the same absorption to make a dough of the proper consistency at the time of mixing. Doughs made from flour of sound wheat had normal handling properties, whereas those of flours from scab infected grain had wet, sticky surfaces, collapsed readily on being removed from the fermentation bowls for punching, and were difficult to handle. The marked decrease in consistency of the doughs made with scab infected flour as fermentation progressed is illustrated by the farinograph curves shown in Figure 1. Loaves made from flours of scab infected grain of Thatcher proofed slightly higher and had very slightly less oven-spring than the loaves representing sound grain. Loaves representing scab infected grain of Ns 2829 proofed slightly less and had less oven-spring than loaves representing sound wheat of this variety. The crust color of loaves from scab infected flours was much darker than the crust of corresponding loaves from sound wheat flours. The crust was thicker, especially on the bottom, and tended to "cup" or pull away from the bottom of the pan. Loaves from Ns 2829 flour were slightly worse in this respect than loaves from Thatcher flour. The crumb color of the loaves from "scab infected" flour was dull and very yellow. It was improved by longer mixing, longer fermentation, or more bromate.

The mean loaf volumes obtained in the second series of baking tests, in which the flours from sound wheat were compared with blends containing 10 and 30% of flours of the same variety from scab-infected wheat, are recorded in Table V, together with an analysis of variance of the data. For all varieties and both fermentation periods combined, the mean loaf volumes for the flours containing 0, 10, and 30% flour from scabby wheat did not differ significantly. For the 2-hr fermentation period, the presence of flour from scabby wheat tended to increase loaf volume, whereas the reverse was the case when the doughs were fermented 3 hr.

The undesirable handling properties of the doughs made entirely of flour from scab infected wheat, and also the darker crust and inferior crumb color of the bread, were greatly minimized in the blends. Even when the blends contained 30% of flour from scabby wheat, the handling properties of the doughs were normal, and the color of the crust and crumb of the bread was influenced only slightly. The loaves of Ns 2829 were the only ones to show "cupping" on the bottom when "scabby" flour was included in the mixture.

From an agronomic standpoint wheat scab may cause serious economic loss to the grower. The small, heavily infected kernels would tend to be lost in commercial wheat cleaning operations and thereby

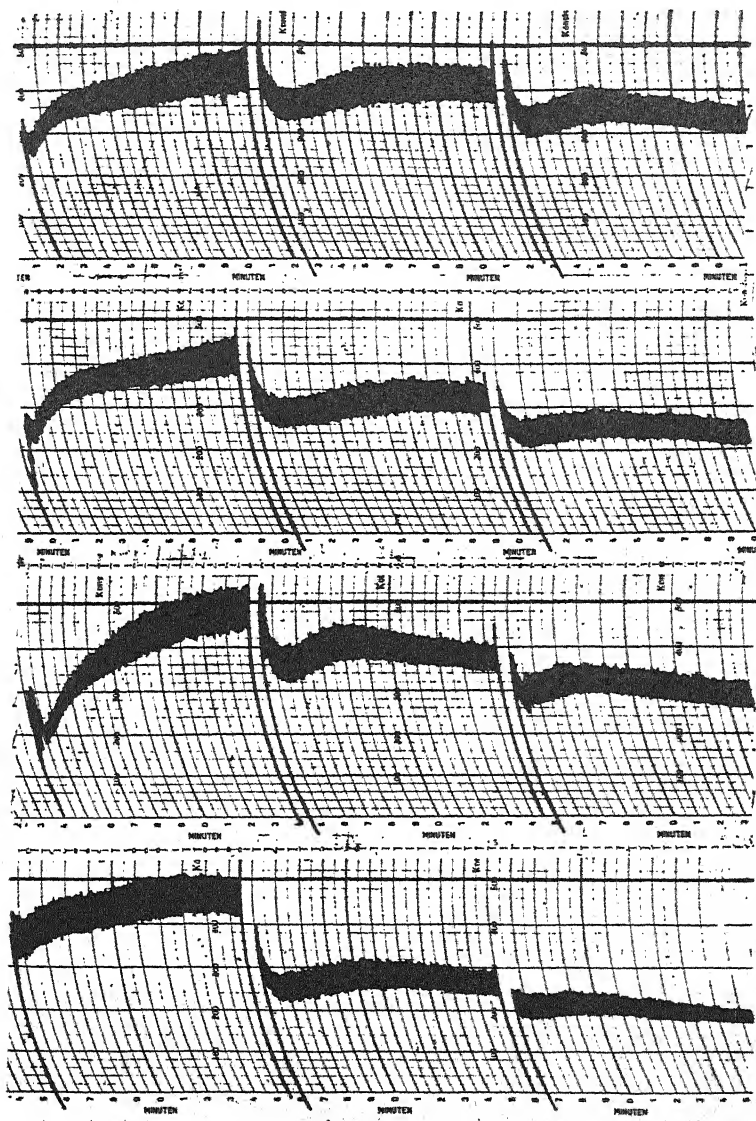


Fig. 1. Farinograms showing the influence of scab infection on the changes in dough mobility during fermentation. In each row, the first curve was obtained upon mixing the dough ingredients, and the second and third curves after one and two hr fermentation, respectively.

Top row—flour doughs representing sound Thatcher  
 Second row—flour doughs representing scabby Thatcher  
 Third row—flour doughs representing sound Ns 2829  
 Bottom row—flour doughs representing scabby Ns 2829



TABLE V

MEAN LOAF VOLUMES FOR FLOUR BLENDS CONTAINING VARYING PROPORTIONS OF FLOURS FROM SCABBY AND SOUND WHEAT OF THE SAME VARIETY

Flour from scabby wheat	Loaf volume for variety <sup>1</sup>				All varieties
	Thatcher	Ns 2829	Regent	Ns 2822	
%	cc	cc	cc	cc	cc
FERMENTATION PERIOD—2 HR					
0	743	813	705	743	751
10	738	870	743	803	789
30	763	830	763	893	812
All blends	748	838	737	813	784
FERMENTATION PERIOD—3 HR					
0	850	855	915	868	872
10	830	923	870	780	851
30	820	883	863	868	859
All blends	833	887	883	839	861
Mean	791	862	810	826	822

<sup>1</sup> A.A.C.C. formula with the addition of 1 mg potassium bromate per 100 g of flour.

## ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	F
Varieties, V	3	17,274	12.2†
Fermentation periods, F	1	56,033	39.6*
Blends, B	2	5,790	4.1
F × B	2	10,103	7.1*
V × F	3	10,875	7.7*
V × B	6	3,237	2.3
V × F × B (Error)	6	1,415	
Between duplicates	24	1,278	
Total	47	10,605	

\* Exceeds 5% point.

† Exceeds 1% point.

reduce the yield of flour per bushel of wheat purchased to a much greater extent than is indicated in these studies; in addition, a somewhat shorter extraction would be necessary in order to produce flours of a given ash content than would be the case with sound wheat. A heavier bleaching treatment would doubtless also be required where scabby wheat is present in the mill mix. The strength of the flour from scabby wheat, as measured by loaf volume, and the oxidation, mixing, and fermentation requirements for optimum baking results

do not differ materially from sound wheat. Since the undesirable handling properties and the inferior crumb color of the bread are largely minimized when the flour from scabby grain is blended with that of normal wheat, scabby wheat does not present a serious problem from the baking standpoint.

### Summary

The effect of scab, caused chiefly by the fungus *Gibberella zeae* (Schw.) Petch (*G. saubinetii* Auct.) on physical and chemical properties, milling, and baking value was studied with four hard red spring wheat varieties—Thatcher, Regent, Ns 2829, and Ns 2822, each of which was separated into sound (scab-free) and scab infected portions.

Scab infection markedly lowered test weight, weight per 1,000 kernels, and also decreased the apparent specific gravity of the kernels.

Flour yield was slightly but significantly decreased by scab infection. Ash and reducing sugar content of the flours milled from the scabby wheats were somewhat higher, and the carotinoid pigment content considerably higher, than that of the corresponding flours milled from sound wheat.

Differential baking tests with flours representing sound and scabby Thatcher and Ns 2829 showed that the absorption at mixing time, and the oxidation, mixing, and fermentation requirements of the flours from sound and scabby wheat were essentially similar. Doughs made with flours from the scab infected wheats had wet, sticky surfaces, collapsed readily, and markedly decreased in consistency as fermentation progressed. These doughs produced bread with a darker crust, and with a duller and more yellow crumb color than the corresponding doughs made with flours from sound wheat; also they exhibited a characteristic tendency to "cup" or pull away from the bottom of the baking pan.

These undesirable baking properties of flours from scabby wheat were greatly minimized when the flours were blended with those from sound wheat. The loaf volumes of blends containing up to 30% of flour from scabby wheat compared favorably with those for flours milled from sound wheat.

### Acknowledgment

The authors are indebted to J. J. Christensen, Division of Plant Pathology, Minnesota Agricultural Experiment Station, for the determinations of the microfloral content given in Table I.

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# THE EFFECT OF VARIATIONS IN CANADIAN SPRING WHEAT ON THE THIAMINE AND ASH OF LONG EXTRACTION FLOURS<sup>1</sup>

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Several investigations of the effects of variety, soil, and climate upon the amount of thiamine in wheat have already been reported. It has been established that, in general, hard wheats are higher in thiamine than soft wheats (Downs and Cathcart, 1941; Sherwood *et al*, 1941). It has also been shown that environmental conditions have a very marked influence upon the thiamine content. Nordgren and Andrews (1941) found that the same variety grown in different locations exhibited great differences in thiamine. Recent studies of winter wheats by O'Donnell and Bayfield (1943) have confirmed the conclusions that thiamine content is influenced by variety, location, and climate. Seasonal variations were found to be considerable; the same varieties grown at the same stations were, on the average, 15% higher in thiamine in 1942 than in 1941. O'Donnell and Bayfield could find no significant correlation between thiamine and protein or between thiamine and wheat ash, but the authors believed that conditions which favored high protein and ash levels also tended to produce wheat high in thiamine.

Geddes and Levine (1942) showed that most of the thiamine of the wheat plant was present soon after blossoming and was thereafter translocated into the developing kernels. The mature kernels contained approximately 77% of all the thiamine in the plant.

Johannson and Rich (1942) conducted a survey of the 1940 Canadian spring wheat crop. Their results gave a mean value of 3.93  $\mu\text{g}$  of thiamine per gram (1.78 mg/lb) with a standard deviation of 0.76  $\mu\text{g/g}$  and a range of 2.2 to 8.0  $\mu\text{g/g}$  (1.00 to 3.63 mg/lb). The means for the three Prairie Provinces showed no significant differences, but samples from Alberta were more variable. No correlation between thiamine and protein was found, though the fact that shrunken kernels were higher in thiamine than starchy kernels suggested that high protein crops would tend to be high in thiamine. They could not relate their thiamine results to soil types.

Studies of the 1939 and 1940 crops carried out by Whiteside and Jackson (1943) proved that the varieties of spring wheat grown in

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Western Canada were significantly different in thiamine content. A statistical analysis of their data also convinced these authors that "thiamine content was influenced by the same environmental factors that influenced protein, kernel weight, and bushel weight."

In Canada, where the sale of flour or bread enriched with synthetic vitamins is forbidden, the government has encouraged the consumption of a flour officially designated as "Vitamin B White Flour." At the end of August, 1943, the definition of this flour was amended to include a maximum dry-basis ash limit of 0.70%, as well as a minimum dry-basis thiamine limit of 400 I.U. per lb (2.64  $\mu\text{g/g}$  or 1.20 mg/lb). Though it is now known that variations in the thiamine content of wheats may not be reflected in the patent flours milled from those wheats, such variations do influence the thiamine of the long extraction flours (about 80%) advocated in official quarters in Canada.

Alcock (1943) has estimated that during the last nine months of 1942, Vitamin B White Flour constituted about 7% of the total volume of flour sold in Canada. Although the annual production of this flour is thus only about 700,000 bbl, its manufacture creates problems for the millers out of all proportion to the quantity involved, since it is made in small lots by mills scattered all over the country. Such operations are, in any case, notoriously difficult to control, and in this instance the difficulties are exaggerated by the opposing demands for low ash and high thiamine. The zone of safety between failure to meet the ash specification on the one hand, and failure to meet the thiamine requirement on the other, may be fairly wide in some mills and nonexistent in others. Differences in milling conditions and in mill equipment are factors, but one of the purposes of this paper is to show that the seasonal and local variations in the amounts of ash and thiamine in available wheat supplies exert an important influence on the problem of milling Vitamin B White Flour.

Aside from these very practical considerations, scientific interest in the natural variations in the thiamine content of wheat, their range, causes, and relationships to other characteristics of the grain, is far from exhausted. No thiamine data have yet been published for the Canadian spring wheat crop of 1941, and, accordingly, while our survey was limited in scope, a brief report of our results will precede the discussion of the problem of milling long extraction flours to ash and thiamine specifications.

### Materials and Methods

Three hundred and eighty-three samples of spring wheat from points fairly well scattered over the three Prairie Provinces were analyzed for protein and thiamine. In addition, samples of Marquis and Thatcher

grown at five stations, and of Red Bobs grown at three of these stations, were supplied to us through the kindness of Dr. C. H. Goulden, Dominion Rust Research Laboratory, Winnipeg. These pure varieties and the products obtained from them by experimental milling were analyzed for thiamine and ash. All the samples were grown in 1941, a year which produced the highest protein crop on record.

Thiamine assays were made by the rapid method described by Hoffer, Alcock, and Geddes (1943), while the milling was carried out on a Buhler experimental mill.

### Results and Discussion

*Results of Thiamine Survey.* Thiamine and protein results for the survey samples, grouped according to provinces, are summarized in Table I.

TABLE I  
THIAMINE AND PROTEIN RESULTS BY PROVINCES FOR WHEAT SAMPLES  
OF THE 1941 CROP

Province	No. of samples	Thiamine			Protein		
		Mean	Range	Std. dev.	Mean	Range	Std. dev.
		$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	%	%	%
Manitoba	93	4.61	3.0-5.5	0.50	14.59	12.4-17.6	1.03
Saskatchewan	182	4.60	2.9-6.3	0.57	15.57	12.2-17.6	0.92
Alberta	108	4.44	3.1-6.1	0.61	14.79	12.1-17.8	1.20
Total	383	4.56	2.9-6.3	0.57	15.11	12.1-17.8	1.13

Histograms showing the distribution of thiamine in all the samples, and for the samples segregated by provinces, are given in Figure 1.

The mean thiamine value for all samples was  $4.56 \mu\text{g/g}$  ( $2.07 \text{ mg/lb}$ ), with a standard deviation of 0.57 and a range of 2.9 to  $6.3 \mu\text{g/g}$  ( $1.32$  to  $2.86 \text{ mg/lb}$ ).

Samples from Manitoba and Saskatchewan had the same average thiamine value, though differing by 1% in protein content. Alberta samples were significantly lower in thiamine. Manitoba samples were the least variable, while samples from Alberta, as was found by Johansson and Rich (1942), displayed the greatest variability. Anderson and Eva (1943), on the basis of data covering a period of 12 years, report that wheat grown in Manitoba is the most uniform and Alberta wheat the most variable with respect to protein content. Such results are not unexpected, since in Alberta, wheat is grown over the widest range of latitude and elevation and on soils which show the widest variability, while in Manitoba the wheat-growing area is much smaller than in the other two provinces.

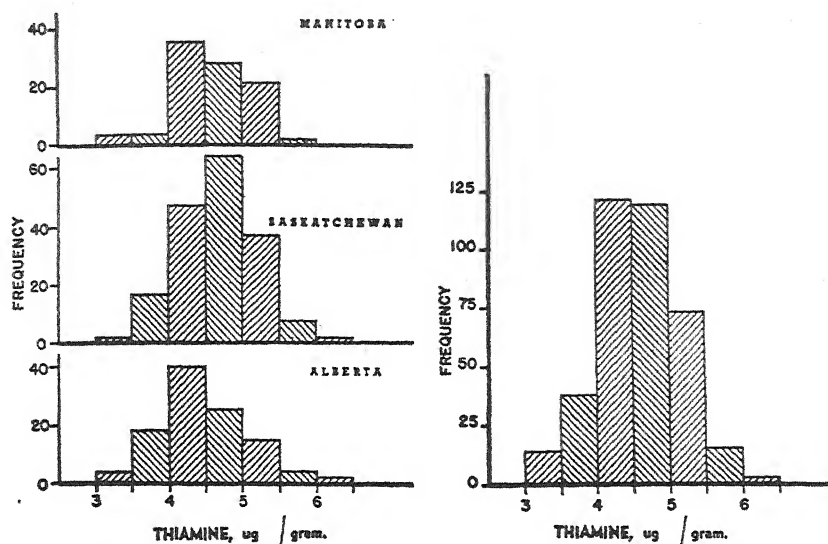


Fig. 1. Frequency distribution histograms for each province and for the three provinces combined, showing the number of samples falling within each 0.5  $\mu\text{g}/\text{g}$  thiamine range.

A map showing the results of the thiamine survey is reproduced in Figure 2.

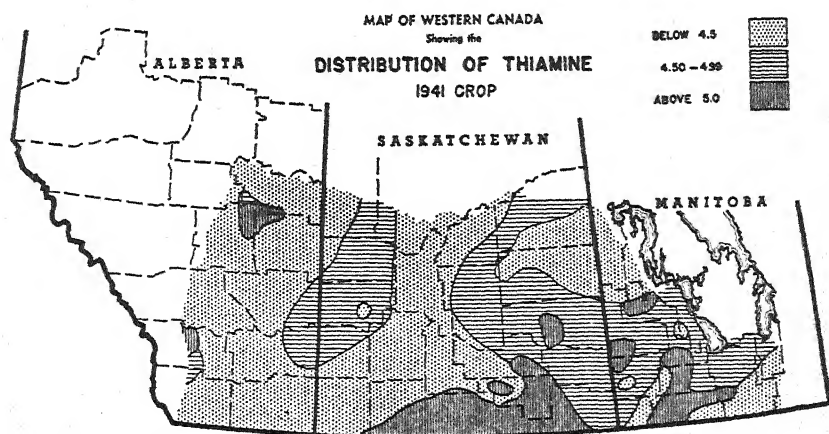


Fig. 2. Map of Western Canada zoned according to the thiamine values of survey samples, 1941 crop.

Because of the small number of samples in relation to the size of the wheat-growing area, the map is very considerably idealized, and it must be admitted that the boundaries of the thiamine zones were by no means as sharp as is indicated. The map should therefore be accepted as an illustration of the fact that samples of different thiamine

content were by no means randomly distributed but were roughly grouped into zones. The thiamine zones showed no relation to soil zones nor to the protein map for the season.

*Thiamine and Soil Zones.* The results for Saskatchewan and Alberta samples were classified according to the soil zones on which the wheats were grown, as shown in Table II.

TABLE II

THIAMINE AND PROTEIN RESULTS FOR SASKATCHEWAN AND ALBERTA SAMPLES OF THE 1941 CROP GROUPED ACCORDING TO SOIL ZONES<sup>1</sup>

Soil zone	No. of samples	Thiamine			Protein		
		Mean	Range	Std. dev.	Mean	Range	Std. dev.
		μg/g	μg/g	μg/g	%	%	%
SASKATCHEWAN							
Black	56	4.50	3.2-6.0	0.51	15.65	12.2-17.6	0.89
Dark brown	59	4.74	3.3-6.3	0.66	15.85	14.2-17.0	0.72
Brown	59	4.59	2.9-5.8	0.56	15.25	13.2-17.4	1.01
ALBERTA							
Black	48	4.48	3.2-5.8	0.61	14.38	12.1-17.8	1.17
Dark brown	37	4.46	3.1-6.0	0.67	15.15	12.9-17.5	1.03
Brown	22	4.34	3.2-5.3	0.48	15.10	12.1-16.7	1.29
SASKATCHEWAN AND ALBERTA COMBINED							
Black	104	4.49	3.2-6.0	0.56	15.07	12.1-17.8	1.21
Dark brown	96	4.63	3.1-6.3	0.68	15.59	12.9-17.5	0.92
Brown	81	4.52	2.9-5.8	0.55	15.21	12.1-17.4	1.09

<sup>1</sup> Eight Saskatchewan and one Alberta sample came from the Gray Soil Zone and hence are not included in the above Table.

The following description of the three soil zones referred to in Table II is taken from a report by Joel *et al* (1936).

"In the southwestern section of the area (Saskatchewan) lies the Brown Soil Zone corresponding to the short-grass prairie region. In this zone the prevailing surface color of the soil is a light or drab brown. The relatively low moisture efficiency has allowed only a short thin cover of natural vegetation, with the result that the amount of organic matter is relatively low.

"The Dark Brown Soil Zone corresponds closely to the intermediate prairie region and the darker color of the surface soil reflects somewhat better moisture conditions and heavier vegetative cover of this region.

"The Black Soil Zone corresponds to the tall-grass prairie region and here the more humid climate and heavy grass growth give rise to dark-colored soils having the highest organic matter content to be found in the Province."

The Dark Brown Soil Zone of Saskatchewan produced wheat significantly higher in thiamine than the other soil zones. When, how-

ever, the results for the three soil zones in both provinces are studied, it becomes apparent that the data show no effect of soil zone on thiamine content. In spite of this, and of the fact that Johansson and Rich (1942) report a similar negative finding, it is probably too early to say that no relationship exists between thiamine and soil zone. A close concordance was found by Anderson and Eva (1943a) between the soil zone map of Western Canada and a protein zone map drawn on the basis of the results of surveys covering a period of 12 years. Nevertheless, in some of the individual years, the outlines of the soil zones could not be detected in the protein maps. More extensive thiamine surveys which reach into the Gray Soil Zone may still reveal a relationship between thiamine and soil zone. The small differences in the average protein levels of our samples from the three soil zones lead to the belief that our data do little to rule out the possibility of such a relationship. We suspect that it does exist, though of course the character of the soil itself may not be the determining factor but rather the continuing climatic differences of which the soil zones are, to a large extent, the consequence.

No explanation can be suggested for the relatively high standard deviation shown by the results of the thiamine determinations on samples from the Dark Brown Soil Zone, especially when it is considered in relation to the low standard deviation of the protein content in this zone.

*Relation between Thiamine and Protein.* The correlation coefficients between thiamine and protein for the three provinces and the three soil zones in Saskatchewan and Alberta are shown in Tables III and IV.

TABLE III

CORRELATION COEFFICIENTS BETWEEN THIAMINE AND PROTEIN BY PROVINCES FOR WHEAT SAMPLES OF THE 1941 CROP

Province	No. of samples	Correlation	1% Point
Manitoba	93	+0.306	0.266
Saskatchewan	182	+0.354	0.190
Alberta	108	+0.338	0.247
Total	383	+0.326	0.132

TABLE IV

CORRELATION COEFFICIENTS BETWEEN THIAMINE AND PROTEIN BY SOIL ZONES FOR SASKATCHEWAN AND ALBERTA WHEAT SAMPLES OF THE 1941 CROP

Soil zone	No. of samples	Correlation	1% Point
Black	104	+0.382	0.252
Dark brown	96	+0.350	0.262
Brown	81	+0.296	0.283



The correlation between thiamine and protein for each of the three provinces and for each of the three soil zones in Saskatchewan and Alberta was significant. The association between thiamine and protein is made still more evident by the arrangement of the data shown in Table V, where all the samples have been grouped according to protein content, and the calculated average thiamine content of each group is given.

TABLE V  
THIAMINE VALUES FOR WHEAT SAMPLES OF THE 1941 CROP GROUPED  
ACCORDING TO PROTEIN CONTENT

Protein %	Thiamine values			
	Man.	Sask.	Alta.	Total
	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
Under 13.9	4.36	4.11	4.26	4.28
14 to 14.9	4.64	4.38	4.21	4.42
15 to 15.9	4.74	4.60	4.65	4.64
16 to 16.9	4.83	4.76	4.65	4.75
Over 17	—	4.98	5.20	5.05

A scatter diagram of thiamine on protein is shown in Figure 3.

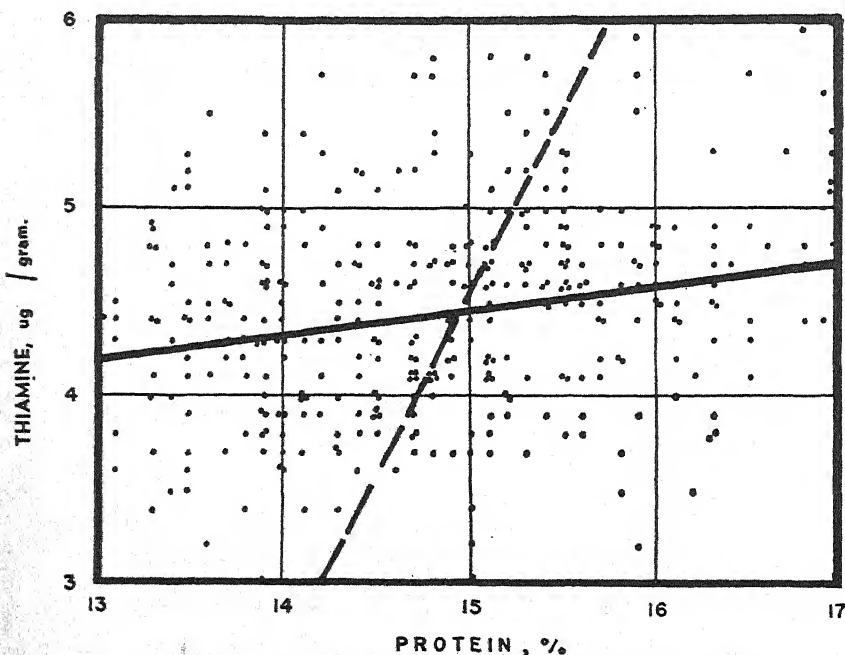


Fig. 3. Scatter diagram for thiamine and protein content in wheat samples, 1941 crop. The solid line is the regression line of thiamine on protein and the broken line the regression line of protein on thiamine. Correlation coefficient is  $+0.326$ .

Because the points are so widely scattered on the correlation surface, it is impossible to predict the thiamine content of a sample of country-run wheat from the amount of protein it contains. The protein in mill mixtures and shipments from terminal elevators, which consist of blends of wheats from many parts of Western Canada, should, however, afford a basis for the prediction of vitamin content.

*Seasonal Variations in Wheat Thiamine.* From the work of Geddes and Levine (1942) it is evident that after blossoming, there is a steady movement of thiamine from the stems, leaves, and glumes of the wheat plant to the growing kernels, with but little net gain in the amount of thiamine in the plant as a whole. In a year of low yield per acre, the plant thiamine is distributed over a smaller number of kernels, or the kernels are thin and shrunken because normal filling has not occurred. In either case, a relatively high thiamine content is to be expected in the seed. On the other hand, the work of O'Donnell and Bayfield (1943), to which reference has already been made, leads to the conclusion that the longer the filling and ripening period, the higher will be the test weight and the lower the thiamine content of the crop. A negative correlation is therefore to be expected between yield per acre and thiamine content. Up to the present, limited studies have been made of only three Western Canadian crops. As far as they go, these studies confirm the expected relationship, as is shown in Table VI.

TABLE VI  
THIAMINE CONTENT AND YIELD OF THREE WESTERN CANADIAN WHEAT CROPS

Year	Mean yield	Thiamine
	<i>bu/acre</i>	<i>μg/g</i>
1940	18.5	3.93 <sup>1</sup>
1941	12.9	4.56
1942	28.6	3.45 <sup>2</sup>

<sup>1</sup> Johansson and Rich (1942).

<sup>2</sup> Average of 236 samples tested in the laboratory of the Western Canada Flour Mills Co., Ltd.

Preliminary data for 1943, when the yield of wheat per acre was 16.7 bu, indicate a mean thiamine content between 3.9 and 4.6  $\mu\text{g/g}$  (1.77 and 2.09 mg/lb).

*Relation between Thiamine and Ash.* Under the latest regulations, the long extraction flour advocated by the Canadian government, called Vitamin B White Flour, must contain, on a moisture-free basis, not more than 0.70% ash and have a natural thiamine content of not less than 2.64  $\mu\text{g/g}$  (400 I.U. or 1.2 mg/lb). Previously, any product which met the legal definition for flour could be packed as Vitamin B White Flour, provided it carried the required amount of thiamine and this thiamine was derived from the wheat kernel. Since the ash

in flour is related to the ash content of the wheat milled (Sherwood and Bailey, 1928), it is evident that the ratio of thiamine to ash in available wheat supplies has become a matter of importance for Canadian millers with the adoption of an ash limit for Vitamin B White Flour.

The regulations referred to were only announced after the work described in this paper had been completed and no attention was given to the ash content of our survey samples. However, ash and thiamine determinations were made on a few samples of pure varieties grown at different stations in 1941, and since these results may now possess some current interest, they are recorded in Table VII.

TABLE VII

ASH AND THIAMINE VALUES AND RATIOS OF THIAMINE TO ASH FOR PURE WHEAT VARIETIES GROWN AT FIVE STATIONS

Station	Marquis			Thatcher			Red Bobs		
	Ash	Thiamine	T/A	Ash	Thiamine	T/A	Ash	Thiamine	T/A
	%	µg/g		%	µg/g		%	µg/g	
Brandon	1.69	4.46	2.64	1.66	4.33	2.61	—	—	—
Indian Head	1.75	4.17	2.38	1.66	4.46	2.69	—	—	—
Melfort	1.28	4.08	3.19	1.35	4.00	2.96	1.24	4.43	3.57
Edmonton	1.40	4.10	2.93	1.45	5.03	3.47	1.27	4.33	3.41
Lacombe	1.44	3.83	2.66	1.30	4.41	3.39	1.34	3.90	2.91

It is clear, even from our very limited data, that the ratio of thiamine to ash in wheat varies widely. A mill grinding such wheat as is represented by the samples from Lacombe, Edmonton, and Melfort might be expected to experience less difficulty in producing long extraction flours to meet thiamine and ash specifications than a mill using wheat represented by the Indian Head and Brandon samples. The Marquis and Thatcher samples for both groups were approximately the same in thiamine content, but in the first group, the ash content was definitely lower—1.37% as compared with 1.69%.

The results of experimental milling tests, using a Buhler mill, bore out these expectations. All the Marquis and Thatcher samples were milled, and ash and thiamine determinations were made on all the mill products, consisting of six flour streams, shorts, and bran from each milling. Regression curves for thiamine on ash, each based on the data for both samples from each station, are reproduced in Figure 4 (a). The fact that all the curves have approximately the same slope shows that the distribution of the total thiamine and ash throughout the kernels is very similar in all five pairs of samples. The low thiamine-ash ratio in the Indian Head and Brandon samples is not, therefore, counterbalanced by a more favorable distribution of thiamine in relation to ash in the kernels.

The problem of the miller is brought out, perhaps more clearly, by a somewhat different treatment of the data. From the ash and thiamine results on the mill products from the two groups of samples—Group (A), consisting of the six samples from Lacombe, Edmonton, and Melfort, and Group (M), the four samples from Indian Head and Brandon—the curves shown in Figure 4 (*b*) were calculated. In these curves the increase in the thiamine content is plotted against the increase in ash content which occurs as the extraction is lengthened. It is realized that if these two mixtures had been milled in a commercial mill instead of a Buhler, curves of different shape would probably have been obtained. Nevertheless, it is believed that the curves would

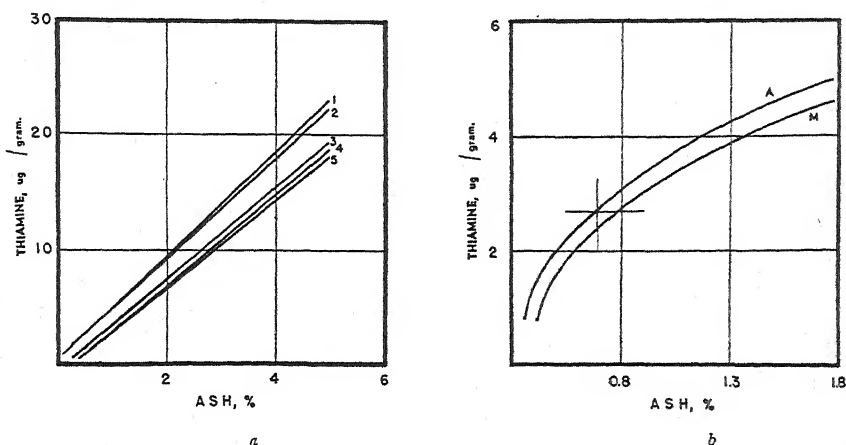


Fig. 4. (*a*) Curves showing the regression of thiamine on ash in Buhler mill streams, obtained by milling Thatcher and Marquis samples from the following five points: (1) Edmonton, (2) Melfort, (3) Lacombe, (4) Brandon, (5) Indian Head.

(*b*) Curves showing the increase of thiamine with the increase in ash content as extraction is lengthened. The point for 0.70% ash and 2.64  $\mu\text{g}$  of thiamine per gram is indicated. Curve (A) includes the samples from Lacombe, Edmonton, and Melfort. Curve (M) includes samples from Brandon and Indian Head.

still have been essentially parallel and that the height above the base line in the critical ash range, about 0.70% (moisture-free basis) as elsewhere, would have been significantly greater in one case than the other.

The territory in Western Canada devoted to wheat growing is large and some mills are obliged to draw their wheat supplies from limited areas. Our results indicate that in a mill grinding wheat having a low thiamine-ash ratio, whether that low ratio is due to low vitamin or to high ash, the production of long extraction flours defined with respect to both ash and thiamine may be a matter of great difficulty, or even an impossibility. Another mill, drawing its wheat from a different territory, may be able to meet the same specifications with ease. That

such differences do occur is already a matter of general commercial experience.

We, ourselves, have a detailed knowledge of only two mills drawing their wheat supplies from different areas. One of the mills is in Winnipeg and draws its supplies mainly from Saskatchewan and Manitoba. Over a period of several months this mill has produced Vitamin B White Flour containing, on a dry basis, 0.685% ash and 2.72  $\mu\text{g}$  of thiamine per gram (1.23 mg/lb). When it is remembered that any decrease in ash requires a sacrifice in thiamine content, and any increase in thiamine can only be obtained by increasing the ash, it will be readily agreed by those who have had experience in mill control and are aware of the uncertainties of the thiamine determination, that these *mean* values indicate an inadequate margin of safety to take care of variations in operating results, sampling errors, and discrepancies in the results of different laboratories. It is scarcely necessary to say, therefore, that on some runs this mill produced flour which contained, at the same time, more than 0.70% ash and less than 2.64  $\mu\text{g}$  of thiamin per gram (1.20 mg/lb). The other mill is located in Calgary and grinds mixtures made up entirely of Alberta wheat. During the same period, its Vitamin B White Flour averaged 0.675% ash and 2.88  $\mu\text{g}$  of thiamine per gram (1.31 mg/lb). Here the margin of safety, though perhaps not adequate, is much wider than at Winnipeg, and according to the control laboratory's data, none of the Calgary flour failed to meet the specifications.

Five representative samples of the wheat mixtures from each mill were milled on the experimental mill. The shorts was divided into two portions by bolting on a 54GG sieve, so that nine samples were obtained from each milling. All 90 samples were analyzed for moisture, ash, and thiamine, and taking average results, the ash and thiamine values for products representing increasing percentages of the Winnipeg and Calgary wheats were calculated. The results are given in Table VIII.

These data support the view that the differences in the Vitamin B White Flour produced at the two mills were due to differences in the wheat. Calgary wheat was higher in thiamine and somewhat lower in ash and, when experimentally milled under the same conditions, yielded, at every level of extraction, products possessing a higher thiamine-ash ratio than Winnipeg wheat. Wheat mixtures showing much wider differences in thiamine-ash ratio could undoubtedly be found in other mills in Western Canada, and it would thus seem that any legal maximum ash limit, for flours milled to meet a minimum natural thiamine requirement, must either be so low as to work a

TABLE VIII

THIAMINE<sup>1</sup> AND ASH<sup>1</sup> RESULTS FOR BUHLER EXPERIMENTAL MILL PRODUCTS FROM COMMERCIAL WHEAT MIXTURES<sup>2</sup>

Winnipeg wheat mixtures				Calgary wheat mixtures			
Extraction	Ash	Thiamine	T/A	Extraction	Ash	Thiamine	T/A
%	%	µg/g		%	%	µg/g	
43	0.51	0.77	1.51	40	0.50	0.79	1.58
59	0.53	1.04	1.96	56	0.51	1.05	2.06
70	0.58	1.57	2.71	69	0.55	1.60	2.91
73	0.60	1.61	2.68	71	0.55	1.64	2.98
78	0.71	2.46	3.46	77	0.66	2.56	3.88
83	0.94	3.10	3.30	82	0.90	3.40	3.78
100	1.82	4.45	2.45	100	1.77	4.84	2.73

<sup>1</sup> Ash and thiamine are on dry matter basis.<sup>2</sup> Short extraction flours from the Buhler mill are relatively high in ash and thiamine.

hardship on some mills, or so high as to provide no control over the color and other grade characteristics of the flour.

### Summary

Thiamine values for 383 samples of hard red spring wheat grown in Western Canada in 1941 ranged from 2.9 to 6.3 µg/g (1.32 to 2.86 mg/lb) with a mean of 4.56 µg/g (2.07 mg/lb).

Alberta samples were significantly lower and more variable in thiamine content than Saskatchewan or Manitoba samples.

A significant positive correlation between protein and thiamine was found.

A negative correlation between mean yield per acre and average thiamine content of Western Canadian wheat crops was indicated by a study of the available data for three seasons.

The relationship of thiamine content to ash content varies widely in wheats grown in different localities. These variations are reflected in the thiamine-ash ratios of long extraction flours, with the result that some commercial mills have considerably more difficulty than others in producing such flours within specified ash and thiamine limits.

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## THE EFFECT OF SOME WETTING AND REDUCING AGENTS ON THE MIXING TIME AND ON THE QUALITY OF BREAD<sup>1</sup>

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It is generally accepted by bakers that good quality bread will result only when the mixing time employed closely approaches the optimum. The optimum mixing time of a particular flour may be too long or too short to fit the shop schedule. Also, a flour with short mixing requirements may be too sensitive to overmixing and a flour with long mixing time calls for a large expenditure of energy. It would therefore be desirable if the optimum for the flour could be changed to meet shop conditions. This investigation was made to study some of the effects of changing the optimum mixing time, by use of various agents, on the quality of bread from flours differing in their normal mixing requirements.

<sup>1</sup> Contribution No. 105, Department of Milling Industry.

### Review of Related Investigations

Swanson and Andrews (1942) showed that surface active agents, notably Aerosol OT (sodium dioctylsulfosuccinate), increased the mixing time. They suggested that these agents caused a certain type of protein denaturation which allowed greater inter- or intramolecular penetration of water. Such modification of the gluten proteins should influence the baking characteristics. They also demonstrated (1943) that surface active agents influence principally the proteins. These authors (1944) further presented some baking data which indicated that the use of Aerosol OT and cysteine (cysteine monohydrochloride) to change the mixing requirement did not injure the loaf volume or texture of bread.

The reduction of the time required to mix a dough to minimum mobility by compounds containing free -SH groups has been demonstrated by several workers. Jørgensen (1936) presented farinograms and baking data showing that 0.1% glutathione greatly affected the physical properties of dough and also had a harmful action on bread quality. Yeast water had similar effects. Potassium bromate tended to reduce the harmful effects. Jørgensen (1936) and Balls and Hale (1936) stated that glutathione activated latent proteolytic enzymes. Later, Balls and Hale (1936a) suggested that glutathione did not activate the enzymes but rather activated the gluten proteins, thus making the proteins more sensitive to enzymatic attack.

Sullivan, Near, and Foley (1936) found that fresh wheat germ and also its water-soluble fraction reduced the time required to mix a dough to minimum mobility but injured the baking quality. Later, Sullivan, Howe, and Schmalz (1936) isolated glutathione from the germ extract and found that this reduced the mixing time and quality of the baked loaf in a similar manner to the glutathione prepared from yeast. Potassium bromate tended to reduce the harmful effects of glutathione.

Freilich and Frey (1939) showed that cysteine and glutathione produced very harmful effects in bread and also that these could be partly overcome by mixing in oxygen. Ziegler (1940, 1940a) demonstrated that  $\text{KBrO}_3$  oxidized the GSH (glutathione) form to the GSSG form and that this oxidation proceeded slowly in a fermenting dough.

Swanson (1940) demonstrated by mixograms (Swanson and Johnson, 1943) that cysteine decreased the mixing time and increased the rate of breakdown. Swanson and Andrews (1944) showed that cysteine and other reducing agents changed the mixogram patterns and that this was proportional to the molecular concentration of these agents. The similarities of mixogram patterns obtained with cysteine and other agents containing -SH groups suggest that these groups are



the causative components. Sodium chloride tended to minimize the effects of cysteine.

Ofelt and Larmour (1940) have also shown that cysteine reduced the mixing time and that the effect was greater with increasing amounts of cysteine. Cysteine did not cause any significant change in loaf volume, but an improvement in the texture was noted. They expressed the view that the action of cysteine was a colloidal effect on the gluten rather than an activation of enzymes.

### General Plan of Investigation

*Materials.* Tenmarq with a normal long mixing requirement and Chiefkan with a shorter mixing requirement were the varieties chosen for these studies. Both flours had 12.6% protein and the flour ash was 0.43% for Tenmarq and 0.45% for Chiefkan. Both flours were composited from several smaller samples of experimentally milled flour. Data given later show that the mixing time of the Tenmarq flour was about normal for that variety, while for Chiefkan flour it was about 1 minute longer than usual for that variety.

*Methods.* Two baking formulas were employed: namely, the malt-phosphate-bromate (MPB) formula of Aitken and Geddes (1934) and a rich formula which included 6% milk solids (Ofelt and Larmour, 1940). The Swanson-Working type mixer used had a speed of 98 rpm. The baking absorption for each flour was 64%. A standard fermentation of 3 hours and a 55-minute proof was used with both formulas. The loaves were baked at 425°F for 25 minutes. Loaf volumes were measured immediately upon removal from oven. Crumb color and texture scores were made the next day after baking. All loaf volumes are the averages of duplicate bakes.

The variables were: (1) substances which influence the mixing time; (2) their amounts; (3) the duration of mixing; and (4) varying amounts of potassium bromate. Two surface active agents were employed, Aerosol OT and sodium lauryl sulfate. Three reducing agents were employed: cysteine; boiled yeast extract, prepared according to the method of Jørgensen (1936); and H<sub>2</sub>S-saturated water, prepared by bubbling hydrogen sulfide through water. All solutions were made on the day of baking and added to the other baking ingredients in the mixing bowl.

### Experimental Results

*Results from MPB Formula.* The effects of wetting and reducing agents on baking results obtained with the malt-phosphate-bromate formula and optimum mixing time are presented in Table I.

TABLE I

EFFECTS OF WETTING AND REDUCING AGENTS ON BAKING RESULTS, EMPLOYING THE MPB FORMULA AND OPTIMUM MIXING TIME

Treatment	Conc.	Mixing time	Loaf volume	Grain texture	Crumb color	Dough characteristics at pan stage
		min	cc	%	%	

TENMARQ						
Check	%	4.0	885	75-o	85cy	Bucky
Aerosol OT	0.06	4.5	845	73-o	85cy	Bucky
	0.12	5.8	820	70-o	85cy	Bucky
	0.24	8.0	750	65-o	85cy	Very bucky
Sodium lauryl sulfate	0.04	4.0	838	78-o	85cy	Bucky
	0.08	4.5	755	70-o	83cy	Soft
	0.16	4.5	625	60-o	80cy	Soft
Cysteine	0.005	2.0	950	83-o	85cy	Very good
	0.010	1.5	950	83-o	85cy	Very good
	0.020	1.2	900	85-o	85cy	Very good
Boiled yeast extract	ml					
	3	2.7	953	80-o	83cy	Very good
	6	2.0	920	75-o	80cy	Very good
	12	1.8	873	65-o	78cy	Soft
H <sub>2</sub> S-saturated water	3	3.5	890	80-o	80cy	Soft and elastic
	6	3.0	885	82-o	80cy	Soft and elastic
	12	3.0	893	82-o	80cy	Soft and elastic

CHIEFKAN						
Check	%	2.3	800	75-o	80cy	Puttylike
Aerosol OT	0.06	3.0	793	77-o	80cy	Puttylike
	0.12	4.0	805	77-o	80cy	Puttylike
	0.24	5.5	780	75-o	82cy	Puttylike
Sodium lauryl sulfate	0.04	2.3	670	70-c	80cy	Puttylike
	0.08	2.3	715	65-c	80cy	Soft
	0.16	2.2	635	60-c	80cy	Soft and sticky
Cysteine	0.005	2.0	815	77-o	82cy	Soft
	0.010	1.6	810	77-o	82cy	Soft
	0.020	1.5	795	75-o	82cy	Soft
Boiled yeast extract	ml					
	3	2.0	780	77-o	80cy	Soft
	6	1.7	750	72-o	80cy	Soft and sticky
	12	1.5	700	60-o	80cy	Very soft
H <sub>2</sub> S-saturated water	3	2.0	775	78-o	80cy	Soft
	6	1.8	795	77-o	80cy	Soft
	12	1.6	770	77-o	80cy	Soft

The mixing times used were those needed to produce doughs of optimum consistency. The addition of increments of Aerosol OT gradually lengthened the mixing time to double that of the check. Sodium lauryl sulfate had only a small effect on the mixing time. Increments of cysteine made the mixing times of Tenmarq and Chiefkan almost the same. Thus the reduction for Tenmarq, for which the mixing time is normally long, was relatively much greater than for Chiefkan, for which the mixing time is normally short. The effects of boiled yeast extract and  $H_2S$ -saturated water were similar to cysteine.

The effects on baking results from the wetting and reducing agents were variable. Sodium lauryl sulfate was unsatisfactory for both flours. This agent had an unfavorable effect on the physical properties of the dough. Increasing the amounts of Aerosol OT produced baking results which were progressively poorer than the checks for Tenmarq and about equal to the checks for Chiefkan. Cysteine produced marked improvement in loaf volumes, textures, and dough-handling properties of Tenmarq, but for Chiefkan, cysteine had less effect. Boiled yeast extract in the smaller amounts produced results similar to cysteine for Tenmarq. For Chiefkan, increasing amounts of this extract were distinctly detrimental. The  $H_2S$ -saturated water produced no marked effect with either flour.

The data indicate that when an agent such as Aerosol OT increased the mixing time for Tenmarq, the results were unsatisfactory, and when the agent such as cysteine decreased the mixing time, the effects were beneficial. Chiefkan was less responsive to these treatments. The treatment of Tenmarq with Aerosol OT, which caused an increase in mixing time, produced pronounced buckiness of the dough similar to that obtained by an excess of certain oxidizing agents.

*Results from Rich Formula.* Various percentages of Aerosol OT and of cysteine together with 2, 3, or 4 mg of potassium bromate were used with the rich formula. Both optimum and constant mixing times were employed. The results from Tenmarq are presented in Table II, and those from Chiefkan in Table III. The figures in Table II show the following: (1) the effect of potassium bromate was minor or obscured by the other substances; (2) Aerosol OT with optimum mixing time produced bread with larger loaf volumes and with equally good textures as compared to the checks; (3) with constant mixing time, Aerosol OT had no apparent effect on the quality of the bread; (4) cysteine with optimum mixing time produced baking results somewhat better than the checks; (5) with constant mixing time, the results with cysteine were increasingly poorer than the checks. The doughs were soft and sticky with very poor handling properties, indicating serious over-mixing.

TABLE II

EFFECTS OF OPTIMUM AND CONSTANT MIXING TIME WITH VARIOUS CONCENTRATIONS OF AEROSOL OT, CYSTEINE, AND POTASSIUM BROMATE ON BAKING RESULTS FOR TENMARQ FLOUR

Treatment	Conc.	KBrO <sub>3</sub>	Mixing time	Loaf volume	Texture grain	Crumb color	Row in Fig. 1
	%	mg	min	cc	%	%	
OPTIMUM MIXING TIME							
Check		2	4.0	883	88-o	85cy	1
		3	4.0	878	88-o	85cy	1
		4	4.0	815	80-o	85cy	1
Aerosol OT	0.06	2	5.0	938	85-o	86cy	1
	0.06	3	5.0	978	85-o	86cy	1
	0.06	4	5.0	925	82-o	86cy	1
	0.12	2	6.0	973	90-c	88cy	1
	0.12	3	6.0	925	90-c	88cy	1
	0.12	4	6.0	925	85-o	88cy	1
	0.24	2	8.0	885	86-o	85cy	1
	0.24	3	8.0	918	86-o	90cy	1
	0.24	4	8.0	930	86-o	88cy	1
CONSTANT MIXING TIME							
Aerosol OT	0.06	2	4.0	865	87-o	85cy	2
	0.06	3	4.0	893	87-o	85cy	2
	0.06	4	4.0	900	87-o	85cy	2
	0.12	2	4.0	890	85-c	84cy	2
	0.12	3	4.0	890	85-c	84cy	2
	0.12	4	4.0	870	87-c	84cy	2
	0.24	2	4.0	825	83-o	80cy	2
	0.24	3	4.0	873	83-o	80cy	2
	0.24	4	4.0	850	83-o	80cy	2
OPTIMUM MIXING TIME							
Cysteine	0.005	2	3.0	940	85-c	80cy	3
	0.005	3	3.0	873	85-c	80cy	3
	0.005	4	3.0	900	82-c	80cy	3
	0.010	2	2.0	905	85-c	80cy	3
	0.010	3	2.0	905	83-c	80cy	3
	0.010	4	2.0	918	80-c	80cy	3
	0.020	2	1.5	853	83-o	80cy	3
	0.020	3	1.5	868	82-o	80cy	3
	0.020	4	1.5	863	80-o	80cy	3
CONSTANT MIXING TIME							
Cysteine	0.005	2	4.0	868	81-o	80cy	4
	0.005	3	4.0	878	81-o	80cy	4
	0.005	4	4.0	870	80-o	80cy	4
	0.010	2	4.0	865	81-o	80cy	4
	0.010	3	4.0	860	82-o	80cy	4
	0.010	4	4.0	873	80-o	80cy	4
	0.020	2	4.0	798	81-o	80cy	4
	0.020	3	4.0	830	82-c	80cy	4
	0.020	4	4.0	815	80-c	80cy	4

TABLE III

EFFECTS OF OPTIMUM AND CONSTANT MIXING TIME WITH VARIOUS CONCENTRATIONS OF AEROSOL OT, CYSTEINE, AND POTASSIUM BROMATE ON BAKING RESULTS FOR CHIEFKAN FLOUR

Treatment	Conc.	KBrO <sub>3</sub>	Mixing time	Loaf volume	Texture grain	Crumb color	Row in Fig. 2
	%	mg	min	cc	%	%	
OPTIMUM MIXING TIME							
Check		2	3.0	783	80-o	85cy	1
		3	3.0	780	80-o	85cy	1
		4	3.0	795	87-c	88cy	1
Aerosol OT	0.06	2	3.5	793	82-o	85cy	1
	0.06	3	3.5	785	83-o	85cy	1
	0.06	4	3.5	835	88-c	88cy	1
	0.12	2	4.0	823	80-o	83cy	1
	0.12	3	4.0	808	86-c	85cy	1
	0.12	4	4.0	818	89-c	88cy	1
	0.24	2	5.0	808	85-o	85cy	1
	0.24	3	5.0	870	87-c	85cy	1
	0.24	4	5.0	803	87-c	85cy	1
CONSTANT MIXING TIME							
Aerosol OT	0.06	2	3.0	778	85-c	85cy	2
	0.06	3	3.0	803	85-c	85cy	2
	0.06	4	3.0	805	85-c	85cy	2
	0.12	2	3.0	800	85-c	83cy	2
	0.12	3	3.0	820	85-c	83cy	2
	0.12	4	3.0	805	80-o	82cy	2
	0.24	2	3.0	795	85-c	80cy	2
	0.24	3	3.0	828	82-o	80cy	2
	0.24	4	3.0	850	80-o	80cy	2
OPTIMUM MIXING TIME							
Cysteine	0.005	2	2.0	748	82-o	83cy	3
	0.005	3	2.0	800	85-c	83cy	3
	0.005	4	2.0	793	83-c	82cy	3
	0.010	2	1.5	775	80-o	82cy	3
	0.010	3	1.5	775	83-o	82cy	3
	0.010	4	1.5	750	80-o	82cy	3
	0.020	2	1.3	695	75-o	78cy	3
	0.020	3	1.3	720	75-o	78cy	3
	0.020	4	1.3	675	75-o	78cy	3
CONSTANT MIXING TIME							
Cysteine	0.005	2	3.0	710	80-o	82cy	4
	0.005	3	3.0	765	80-o	82cy	4
	0.005	4	3.0	765	80-o	82cy	4
	0.010	2	3.0	695	80-o	80cy	4
	0.010	3	3.0	700	80-o	80cy	4
	0.010	4	3.0	715	80-o	80cy	4
	0.020	2	3.0	625	70-o	75cy	4
	0.020	3	3.0	640	70-o	75cy	4
	0.020	4	3.0	660	70-o	75cy	4

It appeared that the longer mixing time resulting from Aerosol OT, even in the presence of the larger amounts of potassium bromate, caused no deleterious oxidation of the dough. This contrasts with the results obtained with the MPB formula and presented in Table I. It is probable that the presence of milk in the rich formula acted as a buffer in minimizing the effects of oxidation consequent upon long mixing. The buffering action of milk was shown by Ofelt and Larmour (1940). When cysteine was present, the 4-minute constant mixing time was too long and produced deleterious effects.

The data for Chiefkan in Table III show the following: (1) the loaf volumes and textures are on a lower level than those presented in Table II, because of the poorer quality of Chiefkan as compared with Tenmarq; (2) no consistent improvement was noted with the use of potassium bromate; (3) Aerosol OT with both optimum and constant mixing time produced better baking results than the checks; (4) the cysteine treatment produced poorer results than the Aerosol OT treatment; (5) with the optimum mixing time, the results were distinctly better than for the constant time.

The handling properties of dough are difficult to describe adequately because they have no accepted standards of measurement. It was noted that when the mixing time of Tenmarq was reduced with cysteine, the dough-handling properties at the pan stage remained very good. When the time had been increased with Aerosol OT, the doughs were bucky. This was more noticeable with the MPB formula which had no milk. The addition of Aerosol OT did not improve the handling properties of the dough, whereas cysteine made the dough more soft and sticky than the checks.

The photographs of the loaves from Tenmarq corresponding to the data in Table II are presented in Figure 1 and similarly the loaves from Chiefkan (Table III) are shown in Figure 2. The four groups of loaves in each figure are arranged in the same order as the four groupings in the tables. The horizontal placings of the loaves in the figures from left to right are in the same order as the vertical arrangements of the data in the tables. The general groupings of the loaves are further elucidated by the legends for each of the figures. Thus by counting the loaves from left to right in any row and the figures in the tables in any group vertically, comparison can be made between the appearance of any loaf and the baking data.

The appearances of the bread obtained from optimum and constant mixing times, as influenced by the addition of Aerosol OT or cysteine, do not differ markedly from the two sets of three check loaves to the left in each figure. The more accurately measured effects of the treatments are indicated by the data given in the tables.

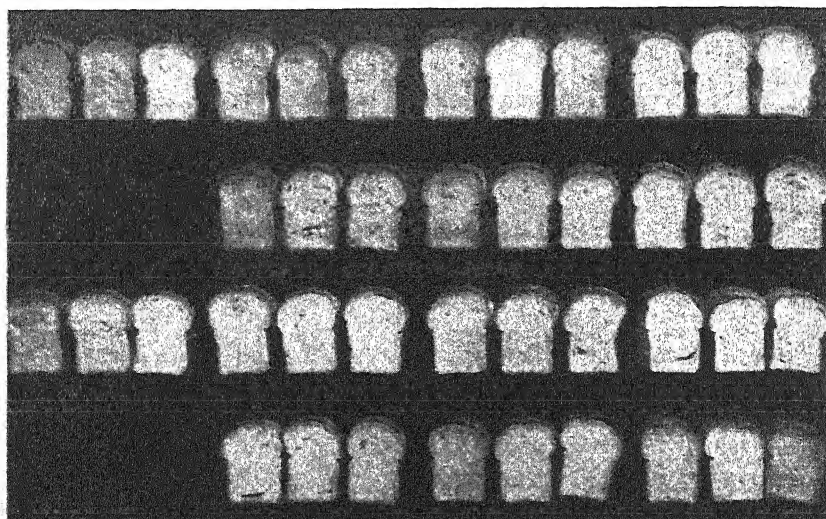


Fig. 1. Effects of optimum and constant mixing time with various concentrations of Aerosol OT, cysteine, and potassium bromate on baking results for Tenmarq flour.

Row 1. Aerosol OT—Optimum mixing time.  
 Row 2. Aerosol OT—Constant mixing time.  
 Row 3. Cysteine—Optimum mixing time.  
 Row 4. Cysteine—Constant mixing time.

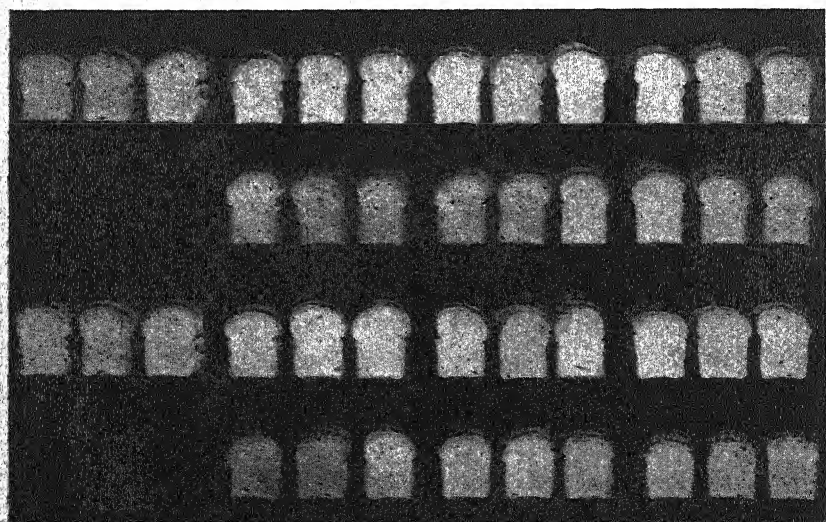


Fig. 2. Effects of optimum and constant mixing time with various concentrations of Aerosol OT, cysteine, and potassium bromate on baking results for Chiefkan flour.

Row 1. Aerosol OT—Optimum mixing time.  
 Row 2. Aerosol OT—Constant mixing time.  
 Row 3. Cysteine—Optimum mixing time.  
 Row 4. Cysteine—Constant mixing time.

### Discussion and Summary

This baking study was made to test the effect on baking of changing the optimum mixing time of two wheat varieties, Tenmarq and Chiefkan, by using surface active and reducing agents. The surface active agents employed were Aerosol OT and sodium lauryl sulfate. The reducing agents employed were cysteine, yeast water, and  $H_2S$ -saturated water. Two formulas were employed, the MPB and a rich formula which included 6% dry milk solids. With the rich formula, the treatments were limited to Aerosol OT and cysteine with varying amounts of potassium bromate.

The results obtained with the MPB formula were different from those obtained with the rich formula, showing that the constituents in the formulas had an influence on the action of the agents used. Sodium lauryl sulfate showed less desirable effects than Aerosol OT. With the MPB formula, Aerosol OT made the dough of Tenmarq bucky, whereas Chiefkan became puttylike. Cysteine with the MPB formula and with a greatly reduced mixing time produced doughs with very good handling properties and improved bread for Tenmarq. With Chiefkan, cysteine made the doughs soft and produced no improvement in the bread.

The effects of boiled yeast extract and  $H_2S$ -saturated water were similar to those from cysteine, indicating that the effective component which shortens the mixing time is the -SH group. Potassium bromate, used only with the rich formula, had small or inconsequential effects. Increasing amounts of potassium bromate did not correct the harmful effects of the larger amounts of cysteine on Chiefkan.

The amounts used of these various agents were critical. When beneficial results were obtained with small amounts, larger quantities often produced harmful effects. Superior results were obtained by using the mixing time which coincided with the point of minimum mobility as affected by the various agents added. The results for this optimum mixing time were consistently better than for the constant mixing time as established by the checks.

Tenmarq was, on the whole, more favorably responsive to these treatments than Chiefkan. No treatments made the characteristics of Chiefkan similar to those of an untreated Tenmarq. Making the mixing time of Tenmarq shorter was beneficial, while shorter mixing time for Chiefkan had deleterious effects. Increasing the mixing time of Chiefkan produced a slight improvement in the bread.

These studies indicate the possibilities of using a surface active agent to increase the mixing time and still obtain good bread. This is conditioned on the use of a suitable formula with proper amounts of



the agent and optimum mixing time. The possibilities are also indicated of using a reducing agent to shorten the mixing time of a flour which normally has a long mixing requirement and in this manner obtained bread of good quality. This is also conditioned by the use of a suitable formula, proper amounts of the agent, and optimum mixing time.

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## EFFECT OF VARIETY AND ENVIRONMENT ON SOME QUALITIES OF MALTED WHEAT FLOUR<sup>1</sup>

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Investigations of malted wheat have recently been reported by Geddes, Hildebrand, and Anderson (1941) and by Kneen and Sandstedt (1942). The latter paper contains extensive references to earlier work, and a further review of the literature seems unnecessary. It is now generally agreed that the utility of malt supplements depends on their alpha-amylase activity and that this varies widely in barley, wheat, and rye malts. No study has yet been made of the difference in the alpha-amylase or other enzymatic activities of malts made from different varieties of hard red spring wheat or from the same variety grown under different conditions; and this matter appeared to merit investigation.

### Materials and Methods

*Materials.* The materials consisted of samples of 23 varieties of hard red spring wheat grown in 1941 under strictly comparable conditions at 16 stations in Western Canada. The varieties are listed in Table I. They represent several named varieties and new crosses which were being studied by Canadian plant breeders and cereal chemists in that year. The stations, also listed in Table I, are fairly widely distributed throughout Western Canada, with five in Manitoba, six in Saskatchewan, and five in Alberta.

From these individual samples two sets of composite samples were prepared, namely, (1) a set of 23 composites, one for each variety, made up by combining equal quantities of wheat from each station, and (2) a set of 16 composites, one for each station, made up by combining equal quantities of each variety.

The varietal composites showed some variation in both bushel weight and protein content. Mean values and ranges were: bushel weight, 64.7 lb, and 63.5 to 66.0 lb; protein content, 15.6%, and 14.9 to 16.2%. The ranges for station composites were 62.5 to 66.5 for bushel weight, and 12.3 to 17.4% for protein content. However, when the lowest value (Fallis, 12.3%) is disregarded, the station range for protein content becomes 14.4 to 17.4%. The high protein values were caused by the dry growing season in 1941. It is unfortunate

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TABLE I

INTERVARIETAL AND INTERSTATION RELATIONS BETWEEN ALPHA-DEXTRINOGENIC ACTIVITY OF AND GAS STIMULATION BY MALT FLOURS

Variety	Gas stimulation	$\alpha$ -dextrinogenic activity	Station	Gas stimulation	$\alpha$ -dextrinogenic activity
	ml	units		ml	units
1. Garnet	163	83	1. Swift Current, Sask.	160	55
2. Red Bobs	161	61	2. Edmonton, Alta.	158	57
3. H44×Reward	160	54	3. Gilbert Plains, Man.	158	53
4. R.L. 625×Mercury, I	158	56	4. Fallis, Alta.	158	51
5. Pilot	153	54	5. Broadacres, Sask.	156	44
6. Marquis×(H44×Marquis), II	152	48	6. Saskatoon, Sask.	155	52
7. Thatcher×Regent	150	49	7. Morden, Man.	154	52
8. Merit 3	150	48	8. Melfort, Sask.	154	51
9. H44×Thatcher, I	150	44	9. Scott, Sask.	154	50
10. Marquis×(H44×Marquis), III	149	45	10. Lacombe, Alta.	154	48
11. Regent Selection I	148	45	11. Winnipeg, Man.	154	48
12. Regent Selection II	148	43	12. Brandon, Man.	151	47
13. Thatcher	146	51	13. Indian Head, Sask.	150	40
14. Red Thatcher	146	36	14. Beaverlodge, Alta.	149	43
15. C.A.N. 1926	145	35	15. Lethbridge, Alta.	148	37
16. H44×Thatcher, II	144	40	16. Portage la Prairie, Man.	140	41
17. Regent×Canus, I	144	37			
18. Renown	142	49			
19. Apex	139	46			
20. Marquis×(H44×Marquis), I	138	46			
21. Marquis	136	41			
22. Regent×Canus, II	134	32			
23. Regent	130	44			

that the materials were not more representative of the average quality of wheat grown in Western Canada.

*Wheat Flour.* The wheats were milled to a yield of 70% in an Allis-Chalmers mill. The flours had an average protein content of 14.9%, and an average ash content of 0.45%.

*Malting.* Duplicate 350-g samples of each composite were malted as follows: steep to 44% at 50°F in equipment described by Anderson and Meredith (1940); germinate and grow for five days at 54°F in equipment not yet described but similar to that of Anderson (1937); and kiln for 24 hours with continuous rise in temperature from 85 to 120°F from 0-6 hours, and a uniform temperature of 120°F from 6-24 hours, in kiln described by Anderson and Rowland (1937). The samples were malted in four successive series, namely, first replicates of variety composites, first replicates of station composites, second replicates of variety composites, and second replicates of station composites; and the order of the samples was randomized within each series.

*Malt Flour.* The malts were milled to a yield of 75% in an Allis-Chalmers mill. The malt flours had an average protein content of 15.2% and an average ash content of 0.76%.

*Analytical Methods.* Protein, ash, gas production (volumetric method; 25 g flour, 6-hour fermentation), Lintner value (diastatic activity), and alpha-dextrinogenic (alpha-amylase) activity, were determined by the standard procedures given in "Cereal Laboratory Methods." Saccharogenic activity was determined by the method of Kneen, Beckord, and Sandstedt (1941). Total Lintner value and total saccharogenic activity were determined by making the original extracts of the flour or malt flour with 1% papain solution as outlined by Sallans and Anderson (1938). The total gas production of the wheat flour was determined by the volumetric method with the addition of papain equivalent to 0.5% of the weight of the flour. The gas stimulation by the malted wheat flour is reported as the increase in the gas production (volumetric method) of a standard base flour which results from the addition of 0.2% of the malt flour. Protein, ash, and gas production are reported on a 13.5% moisture basis, and all other values are given on a dry basis.

### Results and Discussion

The alpha-dextrinogenic activity of the malt flour and its ability to stimulate gas production in a standard dough were studied with both the varietal and station composites. For these properties the intervarietal and the interstation relations can be examined. Data on the remaining properties relate to varietal composites, and illustrate only the intervarietal relations.

*Alpha-dextrinogenic Activity and Gas Stimulation.* The data on these two properties of the malt flour for both varieties and stations are given in Table I. The varieties and stations are listed in decreasing order with respect to gas stimulation.

Under the conditions used in the investigation, 0.2% of malt flour was added to a standard base flour having a gas production of 252 ml in 6 hr. The best variety, Garnet, increased the gas production by 163 ml to 415 ml; the poorest variety, Regent, increased the gas production by 130 ml to 382 ml. It is thus apparent that the range for varieties is comparatively small.

With respect to the station data, the range for gas stimulation is still lower. It is 20 ml over all stations, and if the lowest value is neglected it amounts to only 12 ml. While it is probable that the station composites do not represent as wide a range of environmental conditions as might occur in other seasons, the data certainly suggest that the effect of environment on the gas stimulation by malt flours

is not large. In this connection it may be noted that the Fallis sample, which had the lowest protein content, 12.3%, has essentially the same gas-stimulating activity as the Saskatoon sample, which had the highest protein content, 16.8%. These two samples must certainly have been grown under widely different conditions.

The data for alpha-dextrinogenic activity show greater differences between varieties. Garnet again stands first with an activity of 83 units, and this is more than twice the value for the poorest variety, No. 22, which has an activity of only 32 units. Among the stations the maximum spread is considerably smaller; Edmonton with 57 units, gave the highest value, and Lethbridge with 37 units, gave the lowest.

A relation between gas stimulation and alpha-dextrinogenic activity for both the varietal and station composites is clearly indicated in Table I. The correlation coefficients are 0.71 for varieties and 0.79 for stations, and both are well above the 1% level of significance. Kneen and Sandstedt (1942)—and several earlier workers—have already established the existence of this relation; but it has not previously been shown that it occurs both within and between varieties.

Both our correlation coefficients are appreciably lower than that (0.94) reported by Kneen and Sandstedt. Although theoretical explanations of the difference can be offered, the available data are hardly adequate to support an argument. It should be noted, however, that a correlation of 0.94 suggests that increased gassing power is almost wholly dependent on alpha-amylase activity, and that other factors are of little concern; and this led Kneen and Sandstedt to suggest that determinations of added gassing power and alpha-amylase "appear to be equally reliable for evaluating malts." The data given in the present paper do not support this hypothesis. However, we do not wish to be thought too dogmatic in our interpretation of the available correlation coefficients; it is clear that there is a fundamental relation between alpha-amylase activity and gas stimulation, but it is perhaps a moot question as to how close the relation actually is.

Investigation of causal relations by the correlation method is fraught with many difficulties. An association between two properties may exist in a series of samples produced by progressively changing the processing methods, merely because the change affects both properties in a uniform manner; such an association does not prove a causal relation. An association between two properties may be demonstrated with a set of samples of different varieties grown in the same environment, but may not occur in samples of one variety grown in different environments, or *vice versa*. Under these conditions, with pairs of properties such as gas stimulation and alpha-amylase, failure to obtain a correlation with both varietal and station (environmental)

composites leads to the conclusion that no close causal relation exists. However, when a correlation is found with all three sets of samples—samples produced by different methods, different varieties grown in the same environments, and one variety grown in different environments—there is every reason to believe that a fundamental causal relation exists. It is hazardous to base conclusions on the study of only one kind of samples, or on the study of a miscellaneous collection of samples of unrecorded history.

*Effect of Papain Extraction.* It is well known that the free saccharogenic activity of unmalted barley is low by comparison with the total saccharogenic activity determined by means of a papain extract, and that the total activity is correlated with the saccharogenic activity of the malted barley. These relations were investigated for wheat flour and the corresponding malted wheat flour of different varieties.

In the wheat flour, the free saccharogenic activity was found to be about 10% of the total saccharogenic activity. The mean values over all varieties were 3.1 and 32.6 units. The free activity is of little interest and it does not seem necessary to record the individual values for each variety. Data for total saccharogenic activity are given in Table II, and are discussed in the following section.

TABLE II  
VARIETAL MEANS FOR SACCHAROGENIC ACTIVITIES AND GAS PRODUCTION

Variety No.	Malt flour			Wheat flour		
	Sacch. activity	Beta sacch. act.	Alpha sacch. act.	Total sacch. act. (Papain)	Total Lintner value (Papain)	Gas production
	<i>units</i>	<i>units</i>	<i>units</i>	<i>units</i>	<i>° Lintner</i>	<i>ml</i>
1	33.2	29.1	4.1	38.0	292	292
13	29.9	27.4	2.5	41.6	328	237
7	28.2	25.7	2.5	42.1	325	238
18	28.1	25.7	2.4	40.6	316	258
2	27.7	24.7	3.0	33.5	264	301
14	24.6	22.9	1.7	37.6	296	217
19	23.9	21.6	2.3	38.9	302	273
9	23.3	21.1	2.2	35.2	266	240
21	22.4	20.4	2.0	36.8	289	248
3	22.1	19.5	2.6	32.6	256	268
12	21.4	19.2	2.2	29.3	220	265
11	21.1	18.9	2.2	30.3	278	260
16	20.8	18.8	2.0	30.6	229	275
23	19.9	17.7	2.2	30.5	224	265
5	18.4	15.7	2.7	30.0	225	239
10	17.5	15.3	2.2	27.0	204	232
6	17.3	14.9	2.4	26.8	200	238
4	16.8	14.0	2.8	28.5	214	272
17	16.7	14.9	1.8	29.0	214	254
20	15.6	13.3	2.3	26.9	200	228
22	15.5	13.9	1.6	28.7	216	252
8	15.2	12.8	2.4	27.4	206	269
15	13.8	12.1	1.7	27.1	206	242

The gas production and total gas production (papain) of the wheat flour were also examined. The mean values over all varieties were 261 and 272 ml. Thus a tenfold increase in saccharogenic activity resulting from the addition of papain was associated with an increase in gas production of only about 4%. The data certainly suggest that saccharogenic activity has only a very minor effect on gassing power. However, the evidence is of little value because it seems probable that, in the determination of free gassing power, not only the free beta-amylase, but also that part of the latent beta-amylase liberated by the proteolytic enzymes of the flour, are active in the dough.

The correlation between free and total gassing power was 0.97. In these circumstances it does not seem necessary to record the data for the total gassing power of the individual varieties. The data for the customary determination of free gassing power are given in Table II and discussed in the next section.

*Saccharogenic Activities and Gassing Power.* Data for the saccharogenic activity of the malt flour, and for those parts of it attributed to beta- and alpha-amylase, are given in the left-hand section of Table II. The varieties are listed in order of decreasing saccharogenic activity. Considerable differences exist between the varieties; with respect to each property, the highest value is more than twice as great as the lowest value.

It is interesting to note that Garnet, No. 1, is not only highest in alpha-amylase activity and gas stimulation, but is also highest in beta-amylase activity. Although it is not easy to see it in the data, there is a loose but significant association between beta- and alpha-amylase activities. The correlation is 0.49, which exceeds the 5% level of significance but falls short of the 1% level, 0.51. It appears that in wheat as in barley (Sallans and Anderson, 1939) there is a slight tendency for varieties which are high in beta-amylase to be high in alpha-amylase also. The association is very loose, and has little practical significance.<sup>2</sup>

The data in Table II show that approximately 10% of the saccharogenic activity of the malt flour was due to the alpha-amylase. It thus appears that for most practical purposes the determination of saccharogenic activity may well serve as an adequate measure of comparative beta-amylase activities of wheat malts. In the present study the saccharogenic activity of the malt flour and beta-amylase activity gave a correlation coefficient of 0.996. This fact is worth

<sup>2</sup> Kneen, Beckord, and Sandstedt (1941), in commenting on this matter, write: "Sallans and Anderson (1939) also found a significant correlation between malt-saccharifying and malt-liquefying activities. The data in Table III are not in agreement with this finding." However, a footnote on the same page shows that the correlation coefficient for "the data in Table III" is significant; it is 0.603, which is above the 5% level, 0.576.

recording if only to show that a very high correlation between two properties does not necessarily show that one is dependent entirely on the other, and that a fundamental causal relation does not exist with any additional factor or factors, in this case alpha-amylase activity.

The total saccharogenic activity (papain) of the wheat flour is fairly closely correlated with the saccharogenic activity of the malt flour; the correlation coefficient is 0.88. As would be expected it is more closely correlated with the beta activity of the malt flour for which the correlation coefficient is 0.90. It follows that the comparative saccharogenic activities, and more especially the beta activities, of malt flours made from different varieties can be estimated with moderate accuracy from data on the total saccharogenic activities of the unmalted wheat flours.

Data on total Lintner values (papain) of the wheat flour are also included in Table II. Since these and total saccharogenic activities (papain) are determined by methods which differ mainly in the temperatures of diastasis, it is not surprising that a correlation of 0.97 is obtained. For comparative purposes the methods are essentially interchangeable.

The last column of data in Table II deals with the gas production of the wheat flour. Here again there are appreciable differences between the varieties; the highest gives a value of 301, and the lowest gives 21. Gas production in the wheat flour is not correlated with any property other than the alpha-amylase activity of the malted wheat flour. The correlation is 0.54 which, though low, exceeds the 1% level of significance. Thus it is not likely to be fortuitous. The explanation may be that gas production in the unmalted wheat flour is related to alpha-amylase activity in the same product, and that the latter is related to alpha-amylase activity in the malted wheat flour. Unfortunately this hypothesis cannot be examined as determinations of the alpha-amylase activity of the unmalted flour were not made.

### Summary

Samples of 23 varieties grown at 16 stations in Canada were composited to provide sets of samples representing each variety and each station. The samples were milled, and were also malted and then milled. Properties of the wheat flours and malted wheat flours were examined.

Increases in the gassing power of a standard base flour, resulting from additions of 0.2% of malted wheat flour, varied between 130 and 163 ml depending on the variety, and between 140 and 160 ml depending on the station. Corresponding variations in alpha-dextrinogenic activity were 32 to 83 units for varieties, and 37 to 57 units for stations.



The intervarietal correlation coefficient for these two properties was 0.71, and the interstation correlation was 0.79.

Additional data are reported for the varietal composites only. A very loose association, represented by a correlation of 0.49, was found between alpha- and beta-amylase activities of the malt flour. About one-tenth of the saccharogenic activity of the malts was contributed by the alpha-amylase. Determinations of total (papain) saccharogenic activity and total (papain) Lintner value in the wheat flour are interchangeable; the correlation is 0.97. The total (papain) saccharogenic activity of the wheat flour is correlated with the saccharogenic activity of the malted wheat flour (0.88), and is, as would be expected, more highly correlated with the beta-saccharogenic activity of the malted wheat flour (0.90).

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## DOUGH OXIDATION AND MIXING STUDIES. VI. EFFECTS OF OXIDIZING AGENTS IN THE PRESENCE OF REDUCING MATTER

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The importance of reducing matter as a factor in bread quality has been well established. Sullivan and co-workers (1936) showed that glutathione is present in wheat germ. Quantitative estimations of reducing matter in different grades of flour have been made in our laboratory (Freilich, 1941) and similar results have been reported by Shen and Geddes (1942). It has also been amply demonstrated that the harmful effects of reducing substances such as glutathione may be eliminated by the use of oxidizing agents in dough.

The Farinograph and similar apparatus have been found useful by different investigators in studying the effects of reducing matter in dough. Sullivan (1936) and Ford and Maiden (1938) obtained Farinograph curves indicating a direct effect of glutathione on gluten. Ziegler (1940) observed that increasing increments of glutathione in dough decreased the swelling time, or time to maximum consistency, in the Farinograph. Swanson and Andrews (1943), with the aid of mixograph curves, found that cysteine and other substances may greatly decrease the mixing periods.

Ziegler (1940, 1940a, 1940b) approached the problem with the apparent purpose of explaining the improving effects of bromate in wheat flour doughs, in which glutathione is believed to be the most harmful factor.

In studies on the rate of oxidation of pure glutathione in water solution, he found that at ordinary temperatures bromate oxidized glutathione very slowly, while iodine oxidized it immediately (1940a). Assuming that the action in dough is similar to that in water solution, he concluded that the improving effects of bromate "can be explained by its slow rate of oxidation of glutathione."

Ziegler (1940a) studied the effects of oxidized glutathione in dough. He assumed that "through oxidation by bromate . . . a certain amount of oxidized glutathione will exist in that dough." He found that oxidized glutathione shortened the swelling time (mixing time) and improved loaf volume. He concluded that the improving effects of bromate in dough are due (a) to the suppression of the harmful effect of (reduced) glutathione, and (b) partly due to the presence of oxidized glutathione in dough.

Ziegler (1940b) also studied the oxidation of glutathione by different oxidizing agents, using Farinograph curves to illustrate some of the results. Reduced glutathione produced rapid breakdown of the dough during mixing, suggesting a specific—SH effect. Similar gluten breakdown was produced when using glutathione oxidized with iodine, and partly oxidized with bromate, in fermenting and nonfermenting doughs, showing that flour constituents do not appear to increase the rate of oxidation of glutathione by bromate, and suggesting that "the oxidized glutathione seems to be reduced again in the nonfermenting dough." Very much more persulfate than bromate was needed to produce similar oxidation of glutathione. This leads Ziegler to say, "It would seem that although bromate action in baking can be explained to a certain extent by its oxidation of GSH, the improvement by persulphate is mainly due to action on other factors."

In our work with glutathione (Freilich and Frey, 1939), we have made reference to its immediate or specific effects, observable during dough mixing. The Farinograph curves in Figure 1 show the effects of glutathione during dough mixing and on remixing the same doughs after fermentation. It is evident that glutathione produced changes in consistency, the slope of the curve, and the time required to reach maximum consistency.

The mixing time results for Figure 1 are shown in Table I.

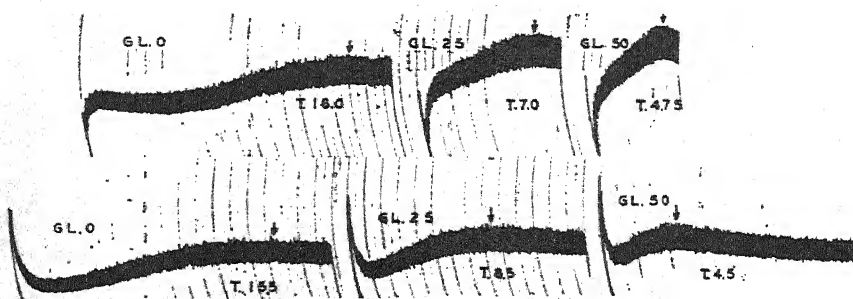


Fig. 1. Effects of varying amounts of glutathione (GL) in dough; the upper curves show the original mixing of the doughs, and the lower curves show the remixing of the same doughs after a normal fermentation period; the point of maximum consistency (T) is indicated by the small arrow above each curve.

TABLE I  
EFFECTS OF GLUTATHIONE ON DOUGH MIXING TIME AND ON REMIXING  
TIME AFTER FERMENTATION

Glutathione per 300 g flour	Mixing time	Remixing time
mg	min	min
0	16.0	15.5
25	7.0	8.5
50	4.75	4.5

These effects of glutathione suggested the possibility of studying the interaction between oxidizing agents and reducing matter in dough. It seemed reasonable to expect that if reducing matter in the dough were oxidized its effects in mixing would be eliminated.

Experiments with glutathione and bromate were conducted accordingly. Table II shows the Farinograph remixing time to maximum consistency and the loaf volume for a series of sponge doughs.

TABLE II  
EFFECTS OF GLUTATHIONE AND BROMATE ON LOAF VOLUME AND FARINOGRAPH  
REMIXING TIME OF SPONGE DOUGHS

Treatment per 300 g flour		Loaf volume	Remixing time
Glutathione	Potassium bromate		
mg	mg	cc	min
0	0	2080	12.75
25	0	1880	6.75
25	5	2160	5.25
25	10	2200	5.0
25	25	2150	4.75

Figure 2 shows the Farinograph remixing curves for these sponges.

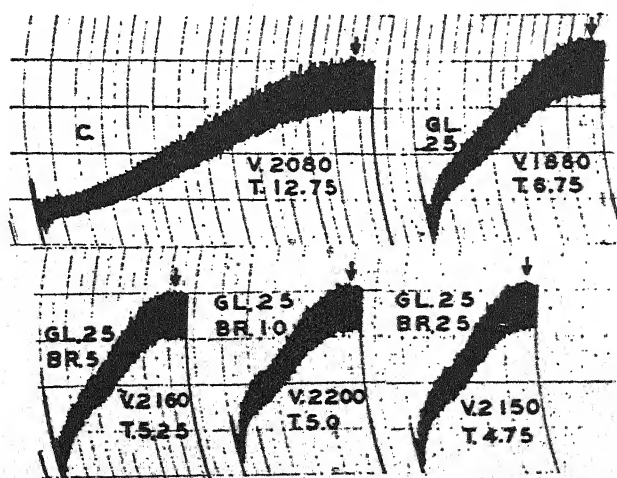


Fig. 2. Remixing curves for sponge doughs (after sponge fermentation), showing the effects of glutathione (GL.) and bromate (BR.) on remixing time (T.); the loaf volume figures (V.) from Table II are also shown; C. is the control curve.

It is evident that 5 mg of bromate was enough to overcome the decrease in loaf volume due to glutathione. It is also apparent that glutathione produced a marked decrease in mixing time, but bromate did not eliminate this mixing time effect; instead, it produced a further decrease in the mixing time, even when using five times the quantity which eliminated the loaf volume effect.

This result indicated the probability that there was no direct oxidation of reducing matter by oxidizing agents in dough, and further study was undertaken in an attempt to learn more about the relationships between the different factors involved in the oxidation-reduction systems in dough.

### Effects of Bromate on Remixing Time of Sponge Doughs

In view of the previous results with bromate, experiments with bromate alone were conducted to note its effects on remixing time.

The results are shown in Table III and Figure 3.

TABLE III  
REMIXING TIME OF SPONGES WITH VARYING AMOUNTS OF BROMATE

Potassium bromate per 300 g flour	Remixing time
mg	min
0	10.0
5	9.0
10	8.5
25	7.25

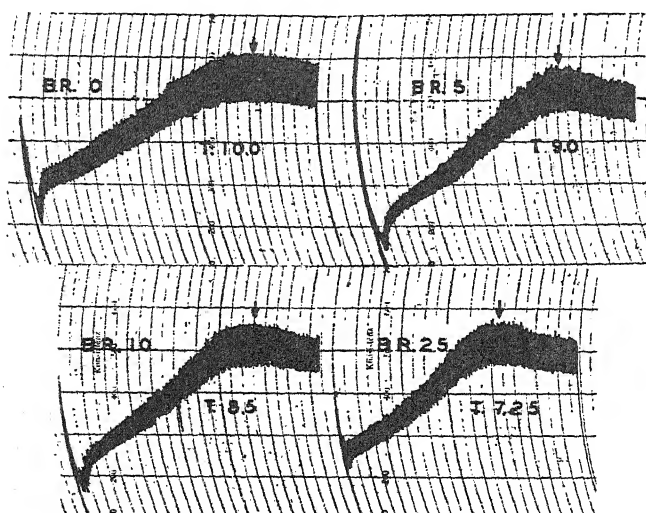


Fig. 3. Effects of bromate (BR.) on remixing time (T.) of sponge doughs.

These results showed that bromate shortens *remixing time* even in the absence of glutathione. This seems to explain the decrease in mixing time beyond that given by glutathione alone, when using both bromate and glutathione in dough. It is, however, apparent that the effect on mixing time of sponge doughs is not very significant when using normal amounts of bromate—5 mg or less per 300 g flour.

### Glutathione and Bromate in Straight Doughs

Straight doughs with glutathione alone and with added bromate were mixed in the Farinograph to note mixing time effects. The results are shown in Table IV and in Figure 4.

TABLE IV  
EFFECTS OF GLUTATHIONE AND BROMATE ON MIXING TIME IN STRAIGHT DOUGHS

Treatment per 300 g flour		Mixing time <i>min</i>
Glutathione <i>mg</i>	Potassium bromate <i>mg</i>	
0	0	20.0
25	0	8.5
25	5	8.5
25	10	8.0
25	25	8.0

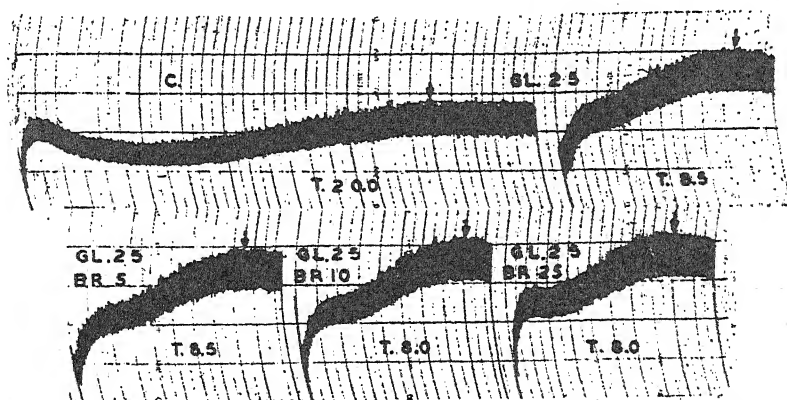


Fig. 4. Effects of glutathione (GL.) and bromate (BR.) in straight doughs; T.—time.

There was a marked reduction in mixing time because of glutathione, but the effects from added bromate under these conditions were negligible. This result was not unexpected, because it has been shown in previous work that the effects of glutathione are immediate, whereas those of bromate are delayed; the development of acidity in the dough as a result of fermentation is a factor which makes the bromate effective. The influence of bromate in straight doughs was observed, there-

fore, by remixing them in the Farinograph after a normal fermentation period; the doughs were originally mixed in the Hobart-Swanson mixer. Straight doughs with bromate and glutathione gave remixing times as shown in Table V and in Figure 5.

TABLE V  
EFFECTS OF BROMATE AND GLUTATHIONE IN STRAIGHT DOUGHS,  
REMIXED AFTER FERMENTATION

Treatment per 300 g flour		Remixing time
Glutathione	Potassium bromate	
mg	mg	min
0	0	16.5
0	50	6.0
25	0	11.0
50	0	6.25
50	50	2.25

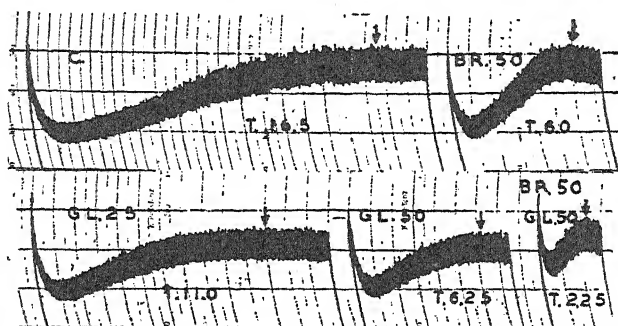


Fig. 5. Effects of bromate (BR.) and glutathione (GL.) in straight doughs remixed after fermentation; T.—Time.

Both bromate and glutathione individually produced great decreases in remixing time, but the reduction produced by their combined effects was far greater than that of either one alone. These results thus confirmed the above findings for sponge doughs.

#### Effects of Oxidized Glutathione and Oxidized Sodium Sulfite<sup>1</sup>

In view of the results thus far obtained, it appeared of interest to study the effects of oxidized glutathione; oxidized sulfite was also tried, because sulfite produces mixing effects in dough like those of glutathione.

Glutathione and sodium sulfite were therefore oxidized with potassium bromate and with hydrogen peroxide in acid solution (2 ml N/1 HCl in about 50 ml water) and then used in doughs which were mixed in the Farinograph, fermented, and then remixed in the Farinograph

<sup>1</sup> Oxidized sodium sulfite is, of course, sodium sulfate; the term "oxidized sulfite" is used here to emphasize the point that the product was prepared in the same manner as the oxidized glutathione.

after fermentation. The amounts of potassium bromate and hydrogen peroxide required were determined by titration and agreed with theoretical requirements. The solutions were neutralized with alkali before mixing the doughs. The results are shown in Table VI and in Figure 6. Also shown in Table VI are the results of a test in which glutathione in water solution was oxidized with iodine before use in the dough, compared to a test in which the same amounts of glutathione and iodine were added to the dough separately, so as to avoid contact between them outside of the dough.

TABLE VI  
EFFECTS OF OXIDIZED GLUTATHIONE AND "OXIDIZED SULFITE" ON MIXING AND REMIXING TIME

Treatment per 300 g flour	Mixing time	Remixing time
	<i>min</i>	<i>min</i>
Sulfite, 10 mg, oxidized with $H_2O_2$	15.0	16.0
10 mg, oxidized with $KBrO_3$	15.5	15.5
Glutathione, 25 mg, oxidized with $H_2O_2$	7.5	10.0
25 mg, oxidized with $KBrO_3$	9.0	9.5
25 mg, oxidized with iodine	9.0	7.25
Glutathione and iodine, added to the dough separately	8.25	6.5

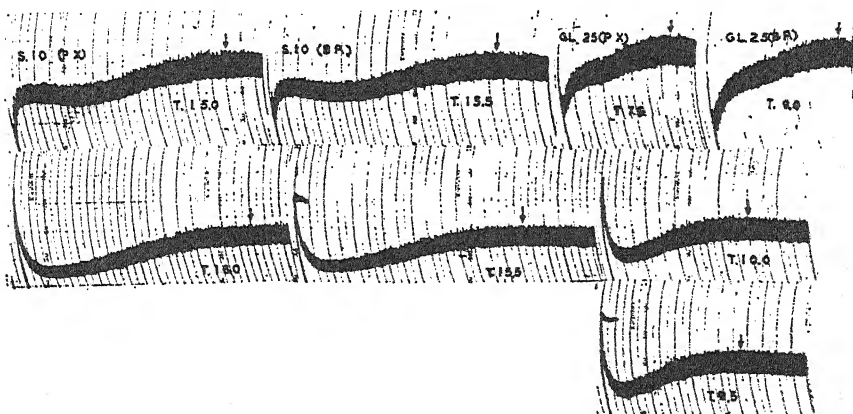


Fig. 6. Effects of oxidized glutathione and "oxidized sulfite" on mixing (four upper curves) and on remixing (four lower curves) of the same straight doughs; S.—sulfite; GL.—glutathione; PX.—oxidized with peroxide; BR.—oxidized with bromate; T.—time.

The treated sulfite acted as though it had been completely oxidized, as will be seen from the results shown. But the glutathione still produced mixing time effects similar to those of reduced glutathione.

In the following experiments, sulfite which was oxidized with iodine was compared to the same quantities of sulfite and iodine added to the dough separately; in the latter instance, direct contact between the sulfite and iodine solutions was carefully avoided before the start of dough mixing. For comparative purposes, the experiment also included a



control; sulfite alone; sulfite oxidized by exposure to air overnight; and sulfite with an excessive amount of iodine, added separately. The results are shown in Table VII and in Figures 7 and 8.

TABLE VII  
EFFECTS OF SULFITE, "OXIDIZED SULFITE," AND SULFITE WITH IODINE (IN THE SAME DOUGH BUT ADDED SEPARATELY), ON DOUGH MIXING AND REMIXING TIME

Treatment per 300 g flour	Mixing time	Remixing time
	<i>min</i>	<i>min</i>
Control	15.5	15.0
Sulfite, 10 mg	9.5	12.5
Sulfite, 10 mg, oxidized with iodine (14.1 ml of 0.01 <i>N</i> ) before use in dough	14.5	14.0
Sulfite, 10 mg and iodine, 14.1 ml of 0.01 <i>N</i> , added to the dough separately	10.75	6.25
Sulfite, 10 mg, oxidized by oxygen (in air)	15.5	15.0
Sulfite, 10 mg and iodine, 30 ml of 0.01 <i>N</i> , added to the dough separately	11.0	2.25
Sulfate (Merck) 12 mg	15.0	15.0

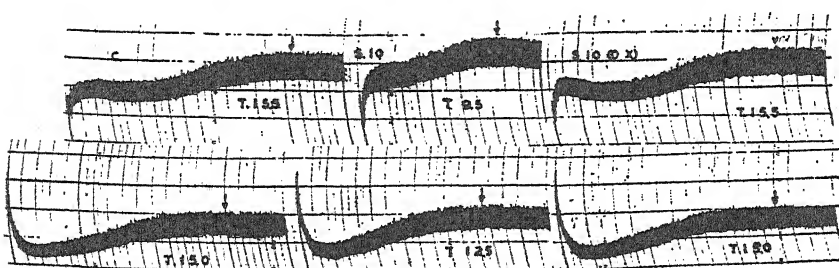


Fig. 7. Effects of sulfite and of "oxidized sulfite" in mixing and remixing; upper curves show original mixing; lower curves show the remixing of the same doughs after fermentation; S.—sulfite; OX.—oxidized in air (see Table VII); T.—time.

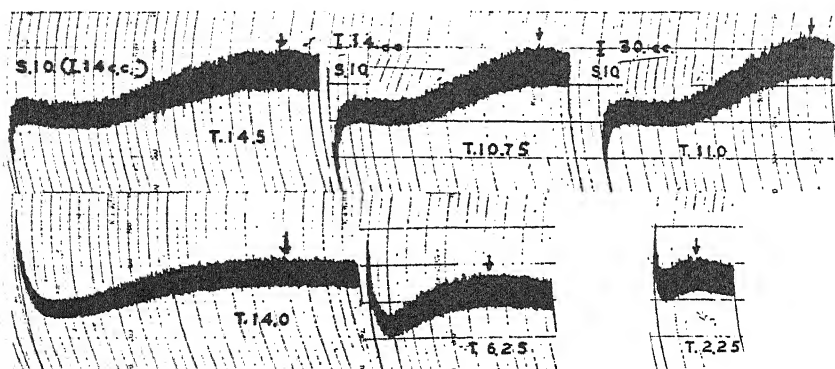


Fig. 8. Effects of "oxidized sulfite" and of sulfite and iodine when added to the dough separately, in mixing and remixing; upper curves show original mixing; lower curves show the remixing of the same doughs after fermentation; S. (I.)—sulfite, oxidized with iodine; I. S.—iodine and sulfite added to the dough separately (see Table VII); T.—time.

These results indicated (*a*) a glutathione effect resulting from sulfite in mixing, and also in remixing, though to a lesser extent; (*b*) the complete oxidation of sulfite by iodine in solution; (*c*) the failure of iodine to oxidize sulfite when they were added to the dough separately;<sup>2</sup> (*d*) the complete oxidation of sulfite by oxygen, and (*e*) an added reduction in remixing time owing to the cumulative effects of sulfite and iodine.

### Discussion

The primary purpose of this paper is to report the results of a study of the effects of oxidizing agents and reducing matter when both are present in a normal dough formula. Our results tend to show that the improving effects of bromate are not due primarily to the oxidation of glutathione. This is contrary to the prevailing viewpoint, as expressed by Sullivan and by Ziegler. Sullivan and co-workers (1936) state that "the oxidizing agents simply change some of the S-H glutathione to the S-S form."

It is obvious that our work confirms a number of the observations made by Ziegler and other workers in the field, but our studies are not in agreement with the general conclusion reached by Ziegler, namely, that the improving effects of bromate are due mainly to the oxidation of glutathione in dough. This viewpoint is retained in his third paper, although some of the work presented there makes its validity appear doubtful.

Our most significant results showed the difference between previously oxidized sulfite in dough as compared to the effect of the same amounts of sulfite and oxidizing agent, but added to the dough batch without previous contact; in the latter instance, there was practically no oxidation of the sulfite in the dough, either during the original mixing or during the fermentation of the dough.

From this it follows that reactions between pure reagents in solution do not necessarily indicate what will happen when the same reagents are present in dough.

Since oxidizing agents overcome the harmful effects of reducing matter on loaf volume and texture without at the same time eliminating the effects of the latter on mixing time, and since there is a difference in effect between "oxidized sulfite" as compared to equivalent amounts of sulfite and oxidizing agent added separately, it is indicated (*a*) that reducing matter may not be oxidized in dough, and (*b*) that reduction and oxidation may involve independent factors in dough. Reducing

<sup>2</sup> This result (*c*) would represent conditions in actual baking practice, where there is no direct contact between the oxidizing agent and any reducing matter that may be present, before the dough is mixed.

agents seem to act on the gluten, while oxidizing agents may act either on another part of the gluten complex, or possibly on some substance associated with it, such as the pentosans described by Baker and co-workers (1943). Reduction tends to soften the dough and make it stringy, while oxidation tightens the dough, though at a later stage in the procedure, when bromate is the oxidizing agent used; the two effects thus counteract each other, and when properly balanced will produce good volume and texture.

Reversible oxidation-reduction systems in dough are indicated (a) by the mixing effects of oxidized glutathione, which is possibly reduced during mixing and remixing, and (b) by the elimination of the "excess bromate" effect in remixing, with a decrease in mixing time, caused perhaps by the reduction of substances oxidized by bromate.

The failure of the common oxidizing agents to combine with added reducing matter in dough is not difficult to understand when it is considered that even under ideal conditions in solution these reactions are not instantaneous. In dough, opportunities for contact between these substances are greatly reduced; in addition, the presence of other oxidizable matter in dough is another factor tending to lessen opportunities for such contact.

### Summary

Reducing matter, among other effects, shortened dough mixing time to a marked degree, as indicated by Farinograph curves. These effects were not eliminated by oxidizing agents, even when present in excessive amounts or in amounts sufficient to overcome the loaf volume reduction due to glutathione.

Oxidizing agents produced a decrease in mixing time after fermentation which was apparently independent of the effects of added reducing matter.

There was a marked difference between the mixing effects of "oxidized sulfite" as compared to the effects of equivalent quantities of sulfite and oxidizing agent added to the dough separately.

Oxidized glutathione was found to produce mixing effects in dough similar to those of reduced glutathione.

Reduction and oxidation probably involve different factors in dough and their effects tend to counteract each other with respect to bread quality.

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#### LABORATORY MALTING. IV. A GERMINATION CHAMBER FOR ROUTINE MALTING TESTS<sup>1</sup>

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The development of laboratory malting equipment on this continent was described by Anderson (1937) when he reported on equipment installed in the National Research Laboratories, Ottawa. This equipment was designed for examining the effect of changes in malting procedure on the evaluation of malts (Sallans and Anderson, 1939 and other papers in the series) with the object of developing a routine method for testing barleys for malting quality. Shortly after the studies at Ottawa were started, the malting equipment built at the University of Manitoba in 1927 was overhauled and details of the changes were published (Anderson and Rowland, 1937). Malting studies conducted at the University of Wisconsin (Dickson *et al.*, 1942) and in Canada (Anderson, Sallans, and Meredith, 1941, and Anderson, Meredith, and Sallans, 1943 and earlier papers in these series) have now resulted in the laboratory malting test becoming a standard tool in

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barley research. Equipment for long-time use has been designed and tested in Canadian laboratories, and an improved germinator for 24 250-g samples is described in this paper.

The new germinator combines the better features of both the earlier Canadian models. It has been in operation for two and a half years in conjunction with a steep tank and kiln already described

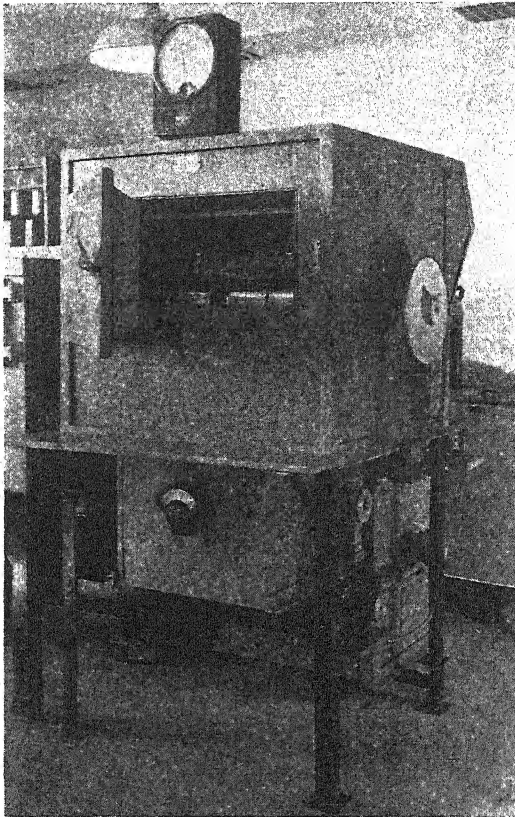


Fig. 1. Exterior view of the germinator.

(Anderson and Meredith, 1940; Anderson and Rowland, 1937). With a 6-day germination period, this equipment produces well-modified malts, with satisfactory malt yield and root loss, and it has proven satisfactory in all respects for routine studies.

### Equipment and Malting Methods

A photograph of the germination unit is shown in Figure 1, and a drawing showing the main working parts is given in Figure 2. The unit

comprises essentially a sample chamber and a conditioning chamber, enclosed in a common case insulated with 4 inches of cork. The whole is protected on the outside by sheet metal and is set inside a  $1\frac{1}{2}'' \times 1\frac{1}{2}'' \times \frac{1}{4}''$  welded angle-iron frame that also serves as a stand.

The sample chamber A is 28'' wide  $\times$  24''  $\times$  24'' and has a rotating brass frame B which carries 24 sample containers. The conditioning chamber C is 15''  $\times$  15''  $\times$  40'' high; the lower portion D is filled with water to a depth of 10 inches, and the top 6 inches is fitted with baffles E.

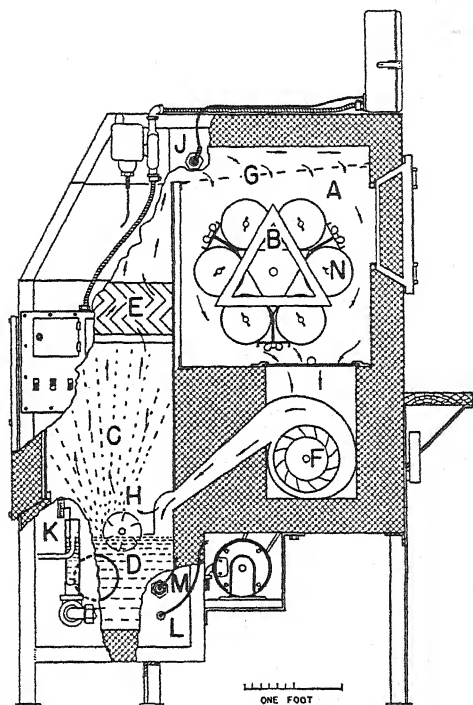


Fig. 2. Sectional view of germinator showing working parts.

Air is drawn continuously from chamber A by the fan F, passed through the spray chamber C where it is humidified, and returned through baffles E and distribution plate G into the sample chamber. The fan is set in a duct below chamber A connecting it with the spray chamber near the water level. The spray, by which air conditioning is effected, is created by a rotor shown at H. The temperature of the water in tank D is controlled by an indicating controller with its sensitive element placed in the germination chamber at J. The water level is maintained by a simple adjustable overflow device K. A dial-read-

ing thermometer, with the bulb L placed in the water tank, provides a means of estimating the relative humidity in the system, by comparing the temperature indicated by this thermometer and that indicated by the indicating controller.

A single  $\frac{1}{4}$  hp motor operates both fan and spray rotor at 1100 rpm by means of V-pulleys to maintain the desired conditions. A  $\frac{1}{4}$  hp  $\text{SO}_2$  refrigeration unit, actuated by the control mentioned above, cools the water. The system is protected against freezing by an auxiliary thermal cut-out, the element of which is indicated at M. A  $\frac{1}{16}$  hp motor operating through a double worm speed reducer and a chain and sprocket assembly rotates the frame B at 3 rp hour (Fig. 1).

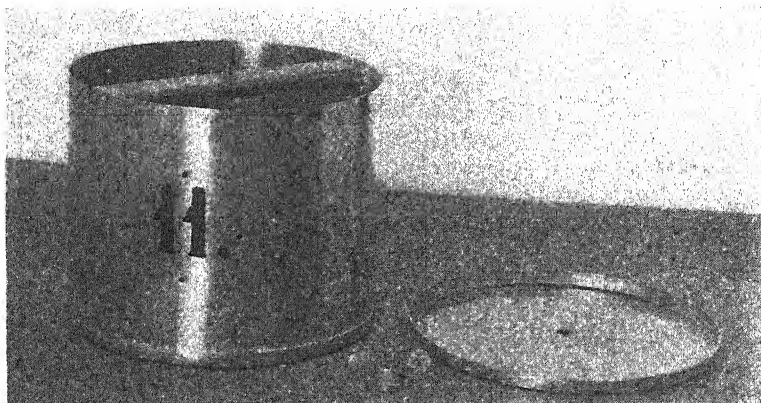


Fig. 3. Sample container.

All materials used for interior construction are corrosion resistant. The lining of the sample chamber is 24-gauge copper as are also the fan duct and baffles. The conditioning chamber is made from 16-gauge brass, brazed at the seams, with a 12"  $\times$  16" inspection plate at the back; the fan is also assembled from brazed brass; and the air distribution plate G is made from  $\frac{1}{8}$ " brass. Shafts are stainless steel and packing glands are brass. The frame that carries the sample containers is made from angle brass; and the spray rotor consists of five 4 $\frac{1}{2}$ " diameter notched-and-bent disks made from 16-gauge stainless steel. All shafts pass through watertight bearings and no air enters the system except when the door is open.

The cages N in which the samples are malted are stainless-steel cylinders, 6 inches in diameter and 5 inches high, with 48 one-eighth inch holes spaced evenly over the cylindrical surface. One of these cages is shown in Figure 3. Various types of cages were tested and it was found that there was excessive loss of moisture from samples in wire mesh containers.

The unit is now operated to produce an air temperature inside the germination unit of 53°F with a relative humidity of approximately 100%. The water temperature and sample chamber temperatures are practically identical, and the capacity of the unit is such that after insertion or removal of samples, with ensuing entry of outside air, operating temperature and humidity are rapidly regained. The temperature of the malts in the cages now in use runs above that in the chamber and reaches a maximum of about 60°F on the third day following the addition of water to the samples.

*Method.* Under routine conditions, the malting laboratory produces 24 malts per week. The malts are made in batches of 12, and the batches are spaced alternately 3 and 4 days apart. By this procedure the steep tank and kiln, which each hold 12 samples, are in continuous use, and the germination chamber always contains two batches of different age.

The samples are steeped to 44% moisture content at 50°F (Anderson and Meredith, 1940), and then transferred to the germination chamber which is maintained at 53°F and 100% relative humidity. After 66 hr in the germinator, water is added to the samples to bring the moisture content up to 46%. The samples are then grown for three more days, making a total of six days in the germinator. They are then transferred to wire mesh cages and placed in the kiln (Anderson and Rowland, 1937) for a 48-hr kilning schedule. Roots are removed from the samples after kilning by kneading in a small bag and sifting the sample.

The system now in use to prevent matting of the samples in the germinator is a combination of the Ottawa and Manitoba procedures. The cages are rotated at 3 rp hour during the day and kept stationary for 12 hr overnight. The malts made in continuously rotating cages were similar to those produced in the Ottawa equipment, in that modification was good, but root losses were somewhat high and malt yield somewhat low. On the other hand, when the cages were stationary, the malts were similar to the original University of Manitoba malts; root losses were very low, malt yield was very high, and there was considerable undermodification.

*Precision.* The germinator has been in use for two and a half years for testing plant breeders' varieties and other laboratory samples. Malts that are satisfactory in modification have been produced from the malting varieties and the reproducibility of the equipment, as measured by differences between duplicate samples, is adequate. The standard deviations for the mean of duplicates for a number of routine investigations carried out in the laboratory are given in Table I.



TABLE I

STANDARD DEVIATIONS FOR MEANS OF DUPLICATES FOR MALT PROPERTIES

Project	No. of varieties	No. of stations	D.F.	Malt yield	Roots	Saccharifying activity	Extract	Wort N.
				%	%	°L	%	%
U. of Alberta	4	3	12	0.14	0.14	1.6	0.12	0.007
Sask. Regional	6	3	18	0.22	0.20	2.1	0.14	0.014
Ont. Regional	4	12	45	0.32	0.25	3.2	0.17	0.019
National Trials	6	5	30	0.21	0.14	1.6	0.17	0.010

A further measure of the reproducibility of the equipment was obtained by making four consecutive replicate batches of malt—preceded and followed by blank batches—from samples of two barleys. The mean values for the malting and malt properties of each batch are given in Table II. The analyses of variance of the data are given in Table III.

TABLE II

MEAN VALUES FOR MALTING AND MALT PROPERTIES FOR EACH BATCH

Property	Batch			
	I	II	III	IV
Weight after germination, g	461.4	460.1	461.7	460.2
Malt yield, %	91.4	91.6	91.5	91.4
Roots, %	4.4	4.3	4.5	4.3
Saccharifying activity, °L	115.7	110.9	110.3	108.5
Extract, %	75.3	75.4	75.3	75.3
Wort nitrogen, %	1.07	1.07	1.06	1.08
Index of nitrogen modification, %	37.2	37.2	36.7	37.6

TABLE III

MEAN SQUARES FOR VARIOUS PROPERTIES

Property	Mean squares			
	Batches	Samples	Ba. X sam.	Within ba.
Weight after germination, g	2.083	0.080	0.307	0.850
Malt yield, %	0.163	0.010	0.277**	0.030
Roots, %	0.083*	0.001	0.003	0.025
Saccharifying activity, °L	114.8**	225.3**	4.2	7.9
Extract, %	0.037	0.227	0.023	0.021
Wort nitrogen, %	0.0012	0.0019	0.0007	0.0005
Index of nitrogen modification, %	1.554	1.172	0.731	0.530
Degrees of freedom	3	1	3	40

\* Denotes significantly greater than mean square due to error within batches. Single signs denote that 5% level of significance is attained. Double signs denote that 1% level of significance is attained.

Differences between batches are very small, except with respect to saccharifying activity, and the interaction errors between batches and samples and the errors within batches are small. The variations in saccharifying activity may be attributed to kilning; saccharifying activity is considerably more sensitive to deviation in kilning temperature than to changes in germination conditions.

### Discussion

The faults and virtues of the equipment originally installed at the University of Manitoba and in the National Research Laboratories, Ottawa, were taken into account in designing the new germinator. The unit resembles the Ottawa equipment in that the air is circulated continuously and is conditioned by means of water sprayed from a controlled bath by a notched-disk rotor, and sample cages are clamped on a rotating frame. It resembles the Winnipeg equipment in that the conditioning chamber and germination chamber are one unit, which minimizes the rise in air temperature between spray and germination chambers and thus facilitates the maintenance of high relative humidity in the latter.

The equipment has sufficient flexibility in the control of temperature, humidity, and air speed to permit studies of malting procedures. An important feature is that there is no bleed-in of air, so that the carbon dioxide content of the air in the chamber is increased by the respiration of the growing samples. When the equipment was built, it was the intention to install an auxiliary carbon dioxide supply, with an automatic control in the chamber, to provide for studies of the effect of malting at different carbon dioxide levels. This development was prevented by the war, but will be kept in mind for future consideration.

The equipment is now installed in the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, together with the steep tank described by Anderson and Meredith (1940), and the kiln described by Anderson and Rowland (1937). These three units are characterized by simplicity of design and sturdy construction, but incorporate many automatic features. They provide the equipment required for routine laboratory malting of 24 samples per week. The malting method has been designed to produce malts similar to those made in commercial plants, and to evaluate the malting quality of new varieties of barley in comparison with the standard variety, O.A.C. 21. The equipment and method fulfill these conditions admirably, and provide a standard malting test of adequate precision for all routine studies.

### Summary

A laboratory germinator with a capacity for growing twenty-four 250-g malts is described. It consists of a sample chamber and a conditioning chamber, enclosed by a common insulated case. Air is circulated continuously through the two chambers. It is humidified and cooled in the conditioning chamber, and maintains conditions of 53°F at saturation within close limits in the sample chamber. Samples are grown in cylindrical containers clamped on a rotating frame. The unit has been in use for over two years, and produces well-modified malts with adequate reproducibility.

### Acknowledgments

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## STORED AND DAMAGED WHEATS FOR STARCH PRODUCTION

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Since about 1941 there has been a tremendous increase in corn utilized by the corn wet-milling industry in the United States. Approximately 75 million bushels annually are normally used by wet

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millers for the production of starch, dextrins, corn sirup, corn sugar, and by-products. In 1942, wet millers ground 130 million bushels. The increased demands for starches and glucose have arisen because of the large quantities required for lend-lease purposes and for essential war industries, and to the virtual cessation of importation of tropical starches. Even though the starch industry has been operating at full capacity, it has not been able to satisfy all the demands for its products. Voluntary rationing of starches, sirups, and other products has therefore been necessary.

The industry has faced the additional problem of an uncertain source of raw material. Grain consumption and distribution have been affected by a combination of war measures leading to a shortage of corn on the cash market. The estimated disappearance of corn for feeding and industrial use during the 1942-43 crop year exceeded the 1943 crop production by more than 175 million bushels. The deficit is being inadequately met by drawing on the carry-over and by rationing to essential users. No relaxation in the demand for corn can be foreseen over the next few years. Exhaustion of the carry-over and a shortage of corn must result unless steps are taken to equalize corn production and disappearance.

Producers or prospective producers of starch or sirups may have to use wheat and other small grains as raw material. Unfortunately, these grains are scarcely more plentiful than corn. Because of the pre-eminent position of wheat as a food and the possible need of exporting large quantities to stricken parts of the world, as well as its recently attained position as an industrial raw material, a liberal stockpile of wheat is a necessity. From the standpoint of economy of foodstuffs, it would therefore seem desirable to obtain starch, whenever possible, from such wheat and other grains as cannot advantageously be used for food or feed.

Although grain stored under the most favorable conditions may retain its milling and baking characteristics for several years, storage for a longer time or under less favorable conditions will not only lower its milling value but also decrease its worth for food or feeding (Jones and Gersdorff, 1941). As of March 31, 1943, there were about 52 million bushels of corn and approximately 245 million bushels of wheat from the crop years of 1938 to 1942 inclusive, owned by the Commodity Credit Corporation. By December 31, these amounts had been reduced to 0 and 116 million bushels, respectively. During this period, all of the aged grain stored on farms, on loan, had been called in, and much of it had been sold. Some of the grain held by the Commodity Credit Corporation, as well as much of that stored privately on farms, may serve as feed, but movement of stored grain to industry, with

consequent release of all current crops for food and feeding, would be preferable from the nutritional standpoint.

Weather, disease, or insects may cause more damage to grain in the field than it would suffer in storage. At marketing centers, approximately 2 to 24% of the wheat inspected is put into "sample grade" because of some type of damage. The amount varies with season and locality. It was conservatively estimated in June 1943 that 2.5% of the wheat on hand in this country was of sample grade. Although this percentage was low, it amounted to 20 million bushels, a quantity which would not be negligible if made available for starch production.

Stored and damaged grains, especially wheat no longer suitable for food or feeding, appear to be logical supplements of corn for the production of starch and starch derivatives. Little or no information is available, however, as to processing difficulties which might be expected. Knowledge concerning the properties of starches from stored and damaged wheats is equally scanty. Studies reported below were therefore carried out on representative unstored sound, stored sound, and disease-, weather-, and heat-damaged wheats, to determine comparative ease of processing, and to characterize the starches obtained. These wheats will hereafter be referred to as "sound," "stored," and "damaged," respectively.

### Experimental

Samples of stored wheat from all known sources and all available types of damaged wheat were obtained in an attempt to get material as old and as severely damaged as possible. Sound, stored, and damaged wheats were obtained from state experiment stations in this country and from Canada. Additional samples of stored and damaged wheats were furnished by the Commodity Credit Corporation and the Office of Distribution of the War Food Administration. The sound wheats included Chiefkan, Kawvale, Triplet, Rex, and Hymar which represent types of wheat that, because of relatively poor milling or baking characteristics or because of the area in which they are grown, are often produced in excess of demand.

*Extraction Methods.* The wheat was thoroughly cleaned before processing, but care was taken not to lessen the degree of damage in stored or damaged wheat samples. The whole grain, rather than flour, was used as raw material for starch extraction. This obviated the necessity for milling quantitatively a large number of small samples. Preliminary experiments had shown that comparable results were obtained when whole wheat and flour were processed.

Laboratory processing methods involving the steeping of wheat, either in water or in a weak solution of sulfur dioxide in water, were

used. Preliminary experiments showed that starch could be more easily extracted after the sulfur dioxide steep. The water steep was used as the basic extraction method, however, since it more nearly simulated commercial wheat starch extraction conditions and avoided possible removal of undesirable odor or color from damaged grain by the sulfur dioxide. A water steep also eliminated the possibility of action on the starch by sulfur dioxide. The acid steep was used for comparative purposes or to process wheats that did not give good yields or white products after a water steep.

The two laboratory processing methods used are described under (1) and (2) below.

(1) A weighed amount of wheat, generally about 400 g, was steeped in cold distilled water at approximately 4°C for 24 hr, ground twice through a Russwin No. 2 handmill with nut-butter cutter, after which it was made into a stiff dough with distilled water and allowed to stand 1 hr at room temperature to permit hydration of the gluten. Starch was then removed by working the more or less coherent mass in successive portions of distilled water. All extraction liquors were combined and worked on No. 17 standard bolting silk to remove pieces of bran and the larger gluten particles with which the starch was contaminated. The partially cleaned starch was recovered from suspension by centrifuging. Finely divided gluten was removed by scraping away the upper layer which formed during centrifuging. This layer also contained small starch granules and, in general, corresponded to the material passing over the end of a commercial starch table. The starch was resuspended in distilled water, centrifuged, and the gluten and other light matter scraped away as before; several repetitions of this procedure yielded a white starch with minimum contamination, which was dried in a mechanical convection oven at 44°C to a moisture content of about 12–15%.

(2) A weighed amount of wheat was steeped in a glass container in a water bath at 44°C for 24 hr in distilled water or in SO<sub>2</sub> solution of known concentration, usually 0.20%. The steep water was continually circulated by a centrifugal pump. At the end of the steeping period, the steep liquor was decanted and the wheat ground in the same manner as described under (1). The ground material was immediately suspended in distilled water and worked on No. 17 standard bolting silk. From this point on, the procedure was the same as described under (1). This is essentially the method described by Slotter and Langford (1943).

Prime-quality starch is obtained by both of these methods. Both methods give recoveries approximating those obtained industrially from wheat.

*Analytical Methods.* Wheat moisture contents were determined by drying the ground grain for 1½ hr at 130°C in a Brabender moisture tester. Moisture of starch was determined by drying a 0.2-g sample to constant weight over P<sub>2</sub>O<sub>5</sub> at 100°C in an Abderhalden drier. Percentage of starch in wheat was determined by a diastase method (Association of Official Agricultural Chemists, 1940). Nitrogen was determined by the Kjeldahl-Gunning-Arnold method (A.O.A.C., 1940) with the modification that the ammonia was received in boric acid solution (Winkler, 1913). The ash content was determined by incinerating the starch at 700°C for 3 hr.

*Viscometric Methods.* Viscosity measurements were made in a Stormer viscometer, using the 100-g weight. Determinations were made at 90°C, using either 2 or 3.5% suspensions of starch in distilled water pasted for 10 min in a boiling-water bath. Eleven readings were taken on each sample, the first immediately after the paste was placed in the viscometer cup, the others at 1-min intervals thereafter. No change was observed during this period when 2% pastes were used. The viscosity of 3.5% pastes decreased, however, during successive determinations; for this concentration the first and eleventh readings are recorded.

TABLE I  
DATA ON EXTRACTION AND PROPERTIES OF STARCH FROM SOUND WHEAT  
(All data given on moisture-free basis)

Wheat					Starch					
Type	Variety	Origin	Starch content	Steep used	Recovery	Nitrogen	Ash	Relative viscosity of paste		
								2%	3.5%	
									Initial	10-min
Soft red winter	Mixture	Commercial	66.7	Cold water	80	0.04	0.16	1.4	3.7	3.3
Soft red winter	Trumbull	Wooster, Ohio	67.5	Cold water	75	0.04	0.14	1.4	3.5	3.0
Soft white	Rex	Colfax, Wash.	69.7	0.2% SO <sub>2</sub>	77	0.04	0.09	1.3	2.3	2.2
(winter)			69.7	Cold water	76	0.04	0.14	1.3	2.3	2.2
Semihard red	Kawvale	Topeka, Kansas	63.8	0.2% SO <sub>2</sub>	75	0.05	0.15	1.3	3.5	2.7
winter			63.8	Cold water	70	0.04	0.16	1.4	2.7	2.4
Semihard red	Triplet	Colfax, Wash.	69.9	0.2% SO <sub>2</sub>	78	0.05	0.15	1.3	2.6	2.6
winter (Western red)			69.9	Cold water	75	0.04	0.15	1.3	2.3	2.1
Hard red winter	Turkey	Minneapolis, Kansas	65.7	0.2% SO <sub>2</sub>	80	0.05	0.17	1.4	3.5	2.8
Hard red spring	Thatcher	Bozeman, Mont., 1941	65.0	Cold water	75	0.04	0.14	1.4	3.1	2.5
		Bozeman, Mont., 1942	66.4	Cold water	80	0.04	0.17	1.3	2.7	2.3
Hard red winter	Chiefkan	Wamego, Kans.	65.5	Cold water	73	0.04	0.13	1.4	2.7	2.4
			65.5	0.2% SO <sub>2</sub>	77	0.04	0.08	1.3	2.4	2.3
White Club	Hymar	Colfax, Wash.	70.1	0.2% SO <sub>2</sub>	73	0.03	0.10	1.3	2.4	2.3
			70.1	Cold water	72	0.04	0.13	1.3	2.4	2.3

*Processing of Sound Wheat.* As a basis for comparison, starches were extracted from several dozen sound hard and soft wheats shortly after harvesting. Representative data are given in Table I. Extractions by both methods are included to furnish a basis for comparison with data in other tables and to show how closely results obtained by the two methods agree. Use of method (1) is indicated in the tables by the words "cold water" in the column headed "steep used."

Each laboratory method yielded a satisfactory product. Recovery of starch usually ran between 75 and 80%. Nitrogen and ash were as low as or lower than the amounts found in commercial wheat starches. In all cases, the starches were white and had no abnormal odors. Viscosities were slightly (0.5 to 1.0 sec) higher than those of commercial wheat starches.

*Processing of Stored Wheat.* About two dozen different samples of wheat, stored (1) under laboratory conditions, (2) in Commodity Credit Corporation bins, or (3) on the farm, were processed in 1943 for starch. All samples were reported to be sound when placed in storage and none had become damaged while stored. Representative data are summarized in Table II.

TABLE II  
DATA ON EXTRACTION AND PROPERTIES OF STARCH FROM STORED WHEAT  
(All data given on moisture-free basis)

Wheat					Starch						
Variety	Source	Crop year	Starch content	Steep used	Recovery	Nitrogen	Ash	Relative viscosity of paste			
								2%	3.5%		
									Initial	10-min	
Thatcher	C.C.C. bin, Jamestown, No. Dakota	1941	%	Cold water	%	%	%	1.3	3.1	2.6	
Marquis	Paskweign, Saskatchewan <sup>1</sup>	1940	61.0	Cold water	80	0.05	0.17	1.4	3.4	2.7	
	Bidora, Saskatchewan <sup>1</sup>	1928	61.7	Cold water	65	0.04	0.15	1.4	3.4	3.0	
	Saskatoon, Saskatchewan <sup>1</sup>	1928	64.1	Cold water	65	0.04	0.18	1.3	2.9	2.6	
Turkey	Clayton, Kansas, farm storage	1928	61.0	Cold water	70	0.04	0.13	1.4	3.2	2.8	
		1927	68.4	Cold water	65	0.05	0.17	1.3	2.6	2.4	

<sup>1</sup> These samples were stored in the laboratory at Saskatoon, Saskatchewan, and supplied to us through the cooperation and courtesy of Dr. A. G. McCalla, University of Alberta, and Dr. R. K. Larmour, University of Saskatchewan.

Even after 14 to 15 years of farm or laboratory storage, wheat gave a good yield of starch. Recovery tended to be somewhat lower from old wheat but was still within the range obtained for new wheats. The lower recovery may have been due to peculiarities of the samples



other than those caused by storage. Available data on the wheats as they entered storage are not sufficient to elucidate this point. The nitrogen and ash contents and paste viscosity of starch from stored wheat were similar to those from new, sound wheat (*cf.* Tables I and II).

*Processing of Damaged Wheat.* More than three dozen samples of damaged wheat were processed for starch. These included musty and sour wheats as well as those injured by weathering, frost, scab and blight, weevil and mold, sprouting, dead germ, incipient heating, and heat. Data on representative samples are given in Table III.

TABLE III  
DATA ON EXTRACTION AND PROPERTIES OF STARCH FROM DAMAGED WHEAT  
(All data given on moisture-free basis)

Wheat			Starch					
Type of damage	Starch content	Steep used	Recovery	Nitrogen	Ash	Relative viscosity of paste		
						2%	3.5%	
							Initial	10-min
Frost damage, 85%	67.0	0.2% SO <sub>2</sub>	70 <sup>1</sup>	0.07	0.11	1.3	3.0	3.0
Very musty	65.1	Warm water	80	0.04	0.14	1.4	3.2	3.2
Weathered in field 8 weeks	64.0	Cold water	80 <sup>1</sup>	0.05	0.13	1.4	3.5	2.7
Scab and blight, 14% (Graded No. 5)	61.3	Cold water	80	0.04	0.16	1.4	3.5	2.8
Weevil and mold, 40%, sour odor	60.0	0.2% SO <sub>2</sub>	80 <sup>1</sup>	0.05	0.08	1.4	3.1	2.7
Sprout damage and incipient heat damage, 58%	65.2	0.2% SO <sub>2</sub>	75 <sup>1</sup>	0.05	0.14	1.4	4.1	3.2
Dead germ, 39%; heat damage, 26%	64.1	0.2% SO <sub>2</sub>	50 <sup>1</sup>	0.08	0.12	1.3	2.6	2.4
Mold and slight heat damage, 49%; weevil, 4%	64.2	0.2% SO <sub>2</sub>	80 <sup>1</sup>	0.04	0.12	1.4	2.9	2.6
Heat damage, 57%; incipient heat damage, 31%; musty odor	63.4	0.2% SO <sub>2</sub>	65 <sup>1</sup>	0.16	0.15	1.2	2.0	1.8
Heat damage, 79%; incipient heat damage, 14%	66.0	Cold water	70 <sup>1</sup>	0.05	0.16	1.4	3.3	2.8

<sup>1</sup> A slightly musty or grassy odor was observed after the starch had been kept in a closed container for several days.

Several of the damaged wheats gave off-color starch when steeped in cold water. In many instances, separation of protein from the starch was difficult. These wheats were therefore processed with a sulfur dioxide steep, as indicated in the table. Not only was the color of the final product improved, but separation of the starch and protein was facilitated. In the case of one heat-damaged wheat, however, considerable difficulty was experienced in removing the gluten from the starch. As a consequence, the final product had a high nitrogen content (0.16%).

Starch was obtained in fair-to-good yield from all samples of damaged wheat studied. Yields were normal from most samples, but low from heat-damaged wheat and from that having dead germ.

All starches were white except those from two badly heat-damaged wheats, which were pale tan, perhaps due to the presence of humins. One of these wheat samples also had dead germ.

Several of the extracted starches had faint unpleasant odors. The odor was noticed after the starch had been kept for several days in airtight containers. Had the starch been dried at a higher temperature and stored in cloth bags, as in commercial practice, practically no odor would be expected. The hot starch pastes had slightly more odor than the unpasted starches.

In general, nitrogen and ash contents as well as paste viscosities were within the limits of those observed for starch from sound wheat (*cf.* Tables I and III). As already mentioned, one starch had high nitrogen content; this starch also had low viscosity.

Microscopically, starch from damaged wheat could not be distinguished from starch prepared from sound wheat. The granules were birefringent and exhibited the typical maltese cross when examined between crossed nicols. The gelatinization temperatures were within the normal range of those from sound wheats.

### Discussion

The experimental data suggest that starches from stored and damaged wheats, excepting those badly heat-damaged or having dead germs, are unchanged and are therefore suitable for industrial use. Although the diversion of stored and damaged wheat to industry would be advantageous from a nutritional viewpoint, it must be recognized that certain factors complicate such a program.

Wheat is stored throughout a wide area in this country. Severe damage likewise is rarely confined to one locality. Accumulation of stored and damaged stocks at the point of processing therefore presents a collection problem.

Dry and possibly wet milling of some damaged wheat would require cleaning of the mill before it could be used for sound grains. Consequent loss of time could be justified only if the mill could operate for a considerable length of time on damaged wheat. In other words, only relatively small mills could afford to handle this grain.

By-products from stored and damaged grain would be expected to be inferior to those from sound wheat. Sourness and mustiness, for example, might remain in the bran and decrease its feed value. The effects of various types of grain damage upon gluten are not known, but storage is reported to have an unfavorable effect upon the value of wheat gluten for food and feed (Jones and Gersdorff, 1941). There is little doubt, however, but that the by-products could be used for other

purposes. Some of these factors would be compensated for by the appreciable discount in price of sample-grade wheat.

For some industrial purposes, grains are utilized for their starch content without the necessity for mechanical separation of the starch. Some cereal flours, for example, are pregelatinized for use as foundry core binders. Whole grain and wheat grits are now used by distilleries for the production of industrial alcohol. Such industries are also outlets for most types of damaged grain in which the starch is sound.

### Summary

Wheat stored for 3 to 15 years in a cool, dry, well-ventilated place in the laboratory or in farm bins is suitable for starch production. Recovery of starch seems to decrease somewhat as the wheat ages. So far as can be determined, the properties of starch are not altered by aging of wheat.

Common types of damage to wheat, other than severe heating, do not render it unfit for starch production. A few types of damaged wheat yield starch having a slight off-odor. Only in badly heat-damaged wheat were the properties of starch found to be modified.

Stored wheats and most damaged wheats appear to be suitable for industrial uses.

### Acknowledgments

All wheat samples were obtained through the Commodity Development Division of the Northern Regional Research Laboratory. The Analytical and Physical Chemical Division furnished analytical data on wheats and starches. Mary J. Cox and Margaret Holzapfel assisted with starch extractions, and Harriet H. Rhodes and Marena Madden made all viscosity determinations. We are grateful to Carol M. Jaeger for supplying information on the grain situation in general.

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# THE AMYLOLYTIC AND PROTEOLYTIC ACTIVITY OF SOYBEAN SEED

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In 1933 Orestano reported that soybean seed contains only beta-amylase. Recently, Newton and co-workers (1939, 1943) observed that the amylolytic activity of soybeans decreases slightly during germination and that it consists mainly of beta-amylase. These workers also described a method for preparing enzyme concentrates.

There is no extensive literature on the proteinase of soybeans. Blagowestschenski and Melamed (1934) found that the soybean contains proteolytic enzymes which digest casein and peptone at pH 5.0.

The investigations recorded in this paper deal with the amylolytic and proteolytic enzymes of soybeans and of germinated soybeans. These enzymes have their own important intrinsic commercial value as does the soybean itself. It is therefore desirable to know more about the enzyme systems involved and their respective activities.

## Amylase Studies

*Materials and Germination Procedure.* Several varieties of soybeans and other grains were used in these studies. For germination, the seeds were soaked in water for 24 hr at 15°C and then grown for 6 and 12 days respectively at 15°C. Precautions were taken to hold bacterial and fungal growth to a minimum, and samples that did become infected were discarded. After germination, the samples were dried in vacuum at room temperature, and in the case of soybeans the material was defatted with petroleum ether.

*Preparation of Extracts for Determination of Amylase Activity.* For these tests the finely ground materials, and in the case of soybeans the defatted beans, were extracted with 20 parts of distilled water for 2½ hr at 20°C, shaking the mixture occasionally. Simultaneous tests were carried out, using papain-cysteine in the extraction for the liberation of the bound amylase according to the technique recommended by Snider (1941). In preparing the amylase concentrates according to Newton *et al* (1939), 250 g of the finely ground seeds and 750 ml of 30% ethanol were placed in a 2-liter flask. The mixture was gently agitated for 10 min in a shaking machine. Then it was pressed through toweling and centrifuged. To the clear fluid, two volumes of ethanol were added. The precipitate was centrifuged off, and in the case of soy-

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beans the yellow color was removed by washing twice with 30 ml portions of ethyl ether. The precipitates were dried in vacuum at room temperature.

*Methods.* The methods developed by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941) for measuring the amylase activities of malt were employed in these studies.

*Saccharogenic Activity* (Kneen and Sandstedt, 1941). The degree of saccharification occurring in 15 min was determined, using 20 ml of 2% Lintner starch buffered with acetate buffer to pH 4.7, plus extract from the material tested and sufficient water to give a total volume of 30 ml. *Saccharogenic units* were calculated as the number of g of soluble starch converted to maltose in one hr at 30°C by one g of dry material tested. As indicated in the method, a starch-extract "blank" was also run, and precaution was taken not to exceed 40% conversion of starch to maltose.

*Alpha-Dextrinogenic (Alpha-Amylase) Activity* (Sandstedt, Kneen, and Blish, 1939). The rate of dextrinization of Lintner starch, buffered with acetate buffer to pH 4.7, was measured in the presence of an excess of beta-amylase, as indicated by the appearance of a "red-brown" color which the reaction mixtures gives with iodine. *Alpha-amylase units* were calculated as the number of g of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized in one hr at 30°C, by one g of the dry material tested. Sandstedt and co-workers found in numerous determinations on malt that the presence of an excess of beta-amylase in this test causes the rate of dextrinization to be 5.4 times as rapid as it would be when the same amount of alpha-amylase acted alone. Hence a "true" measure of the alpha-dextrinogenic activity of malt would be secured by dividing the alpha-amylase units by 5.4.

*Beta-Saccharogenic (Beta-Amylase) Activity.* This activity was calculated from the saccharogenic and alpha-dextrinogenic activities according to the method of Kneen and Sandstedt (1941) and expressed as *beta-amylase units* indicating the number of g of soluble starch converted to maltose in one hr at 30°C by the beta-amylase of one g of dry material tested. Kneen and associates have found on a number of malts tested that the degree of saccharification, due to beta-amylase alone, ranges from 74 to 94% with an average value of 84%. The remaining saccharification was found to be due to alpha-amylase. It is obvious that where alpha-amylase is lacking, the saccharogenic activity may be considered as being due entirely to beta-amylase.

*Dextrinogenic Activity* (Kneen, Beckord, and Sandstedt, 1941). This activity was determined under the same conditions used for alpha-dextrinogenic activity with the exception that the addition of an excess

of beta-amylase was omitted. *Dextrinogenic units* were calculated as the number of g of soluble starch dextrinized in one hr at 30°C by one g of dry material tested.

TABLE I  
AMYLOLYTIC ACTIVITY OF SOYBEAN AS COMPARED WITH BARLEY AND WHEAT

Exp. No.	Grain	Saccharo- genic activity	Alpha- dextrinogenic (Alpha- amylase) activity	Beta- saccharogenic (Beta- amylase) activity	Dextrino- genic activity
		<i>units</i>	<i>units</i>	<i>units</i>	<i>units</i>
1	Soybean A	15.7	trace	15.7	trace
2	Soybean A	16.3	trace	16.3	trace
	(Germinated 6 days)				
3	Soybean A	17.5	trace	17.5	trace
	(Germinated 12 days)				
4	Barley	7.5	trace	7.5	trace
5	Wheat	6.4	trace	6.4	trace
6	Soybean concentrate	201.5	6.4	201.0	6.8
7	Barley concentrate	210.9	4.8	210.7	4.6
8	Wheat concentrate	235.6	4.3	235.4	4.4

*Discussion of Results.* Table I presents values obtained for amylolytic activity of ungerminated and germinated soybeans, and also for a concentrated soybean extract. For comparison, similar determinations were made on barley, wheat, and concentrated extracts from barley and wheat. There is no published method available for measuring very small amounts of alpha-amylase such as may be present in ungerminated grain. The methods employed in the present study for measuring alpha-dextrinogenic activity and dextrinogenic activity gave results which confirm the findings of other workers to the effect that soybeans, like barley or wheat, contain very little, if any, alpha-amylase. However, it was found that germination does not increase either the alpha-amylase or the dextrinogenic activity of soybeans, and in this respect soybeans differ from barley and wheat. In view of the fact that alpha-amylase is practically absent, the saccharifying activity of soybeans appears to be entirely due to beta-amylase; hence, beta-saccharogenic activity is identical in this case with saccharogenic activity.

The soybean concentrate manifests amylase activities similar to that of barley and wheat concentrates. The dextrinogenic and alpha-dextrinogenic activities of the concentrates were measurable and were found to have almost identical values, which was due to the fact that both were determined in the presence of an excess of beta-amylase. However, we are inclined to assume that this measurable rate of dextrinization found in the concentrates is caused rather by the saccharifying activity than by the dextrinizing activity of the concen-

trated extracts. In other words, the alpha-amylase is also here present only in traces and could not be estimated by the methods used in this study. In view of the fact that alpha-amylase activity was found to be negligible, under the conditions tested, the saccharogenic activities of the concentrates were also practically identical with their respective beta-amylase activities.

The saccharogenic activity was also determined on extracts prepared with water and with papain-cysteine from ungerminated and germinated soybeans, and compared with those prepared from ungerminated and germinated barley and wheat. The results of these tests are recorded in Table II. It will be noted that, in contrast to barley and

TABLE II  
THE EFFECT OF PAPAIN-CYSTEINE ON SACCHAROGENIC ACTIVITY  
(pH 4.7—Acetate buffer)

Exp. No.	Nature of material	Saccharogenic units	
		Extraction with water	Extraction with papain-cysteine
1	Soybean A	15.7	16.4
2	Soybean A, germinated 6 days	16.8	16.6
3	Soybean B	13.2	13.4
4	Soybean B, germinated 6 days	13.8	13.8
5	Barley A	7.5	17.6
6	Barley A, germinated 6 days	18.0	24.7
7	Barley B	5.2	13.6
8	Barley B, germinated 6 days	14.2	19.2
9	Barley C	4.6	13.1
10	Barley C, germinated 6 days	14.4	19.5
11	Wheat A	6.0	20.1
12	Wheat A, germinated 6 days	16.8	24.7
13	Wheat B	6.4	19.6
14	Wheat B, germinated 6 days	12.9	20.7

wheat, soybeans showed very little change in saccharogenic activity either in the course of germination or due to the action of proteolytic enzymes. In this connection it is assumed that the proteolytic enzymes present in the soybean itself exert little action on liberation of amylase in the course of  $2\frac{1}{2}$  hr of extraction with water at 20°C. When the results obtained for the water extracts and for the papain-cysteine extracts from ungerminated soybeans are compared with those secured from ungerminated barley and wheat, it is apparent that the beta-amylase present in soybeans is in a "free" state, while that present in barley and wheat is largely in a "bound" state, and is released from this bound state by the papain-cysteine action.

The increase in saccharogenic activity of barley and wheat during germination is due not only to the liberation of bound beta-amylase, but also to the development of alpha-amylase.

The optimum pH range for saccharogenic activity of the soybean was found to be between pH 5.18 and 6.38 with an apparent maximum value close to pH 5.9 (Fig. 1).

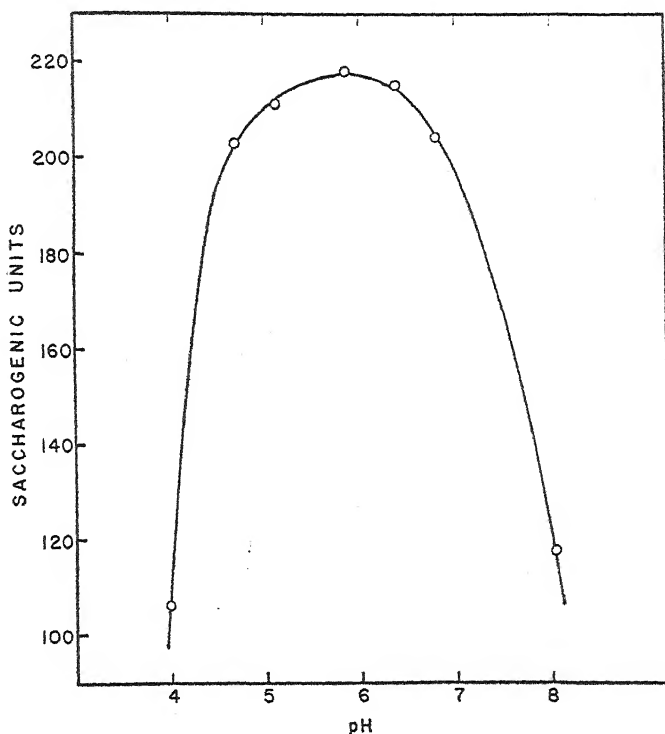


Fig. 1. Effect of pH on saccharogenic activity of soybean amylase concentrates. Saccharogenic units: g of soluble starch converted to maltose in 1 hr at 30°C by 1 g of concentrate.

Judging from the limited number of samples tested, the potential or total saccharogenic activity of soybeans appears to be of the same order as that of barley or wheat; however, since all of the soybean beta-amylase is free, a simple water extract shows much more saccharogenic activity. For convenience, these comparative tests were made at pH 4.7, which is the optimum for barley and wheat amylase but considerably below the optimum for soybean amylase. If determined at the latter pH (5.9), the values for saccharogenic activity of soybeans would be increased by 8-10%.

### Protease Studies

*Method.* In measuring the proteolytic activity of the soybean extracts, a method was employed that had been used successfully in this laboratory in several studies of a similar nature. The method was



essentially the Willstätter (1926) method, using an alkali titration of carboxyl groups in alcoholic solution as modified by Linderström-Lang and Sato (1929). In preparing the proteolytic digest, 10 ml of either a 12% gelatin solution or a 6% casein solution which had been adjusted to pH 7 with citrate buffer, and 10 ml of the extract from the samples were mixed thoroughly in a corked test tube, both solutions having been attempered to 37.5°C. Two drops of toluene were added to the tubes, and they were then digested for 24 hr at 37.5°C. Before and after incubation, 2-ml aliquots were pipetted from each mixture to a titration flask containing 10 ml of 95% ethanol which was sufficient to stop enzyme action. The alcohol mixture was titrated in the presence of 0.1 ml of 0.1% alcoholic solution of ortho-cresolphthalein with 0.05*N* alcoholic KOH to a definite pink color. Twenty ml of boiling ethanol was then added, and the titration was carried again to the definite pink color. The increase in titration of the sample during the 24 hr incubation period represents the measure of proteolysis.

*Extract Preparation.* In extracting the proteolytic enzymes from the samples, several extraction media were used; namely, water, 5% sodium chloride solution, 30% ethanol, and 30% and 50% glycerol. To 20-g samples of the ground defatted beans, 80 ml of each of the respective extraction fluids were added. The extraction was carried on with occasional shaking for 24 hr at 30°C, using toluene as an antiseptic. At the end of this period the mixture was centrifuged, and the clear extract subjected to the test method. Extraction of the proteolytic enzymes with water proved to be inefficient, giving titration values of only approximately 0.1 ml of 0.05*N* alkali solution. Extraction with 30% and 50% glycerol yielded the most active extracts. The values reported in Table III and Figure 2 were obtained with 50% glycerol extracts.

*Nature of Soybean Protease.* No attempt was made in these studies to isolate the various components that are no doubt present in the

TABLE III  
EFFECT OF GERMINATION ON SOYBEAN PROTEASE  
(pH 7.0—Citrate buffer)

Experiment No.	Type of material	Proteolytic activity expressed in ml <i>N</i> /20 KOH per 10 g		
		Soybean variety		
		Y	I	C
1	Soybean, original	1.63	1.37	1.21
2	Soybean, germinated 6 days	4.03	2.95	2.43
3	Soybean, germinated 12 days	6.86	5.85	5.39

soybean protease system. In this discussion, the term "protease activity" includes depeptidases, and polypeptidases, as well as proteinases. For this system, we propose the name "Soyin."

*Discussion of Results.* Experiments recorded in Table III show that during germination there is a very considerable increase in proteolytic activity. Three types of soybean, "Y," "I," and "C," indicate some variation between soybean varieties, but the increase is comparable in all cases.

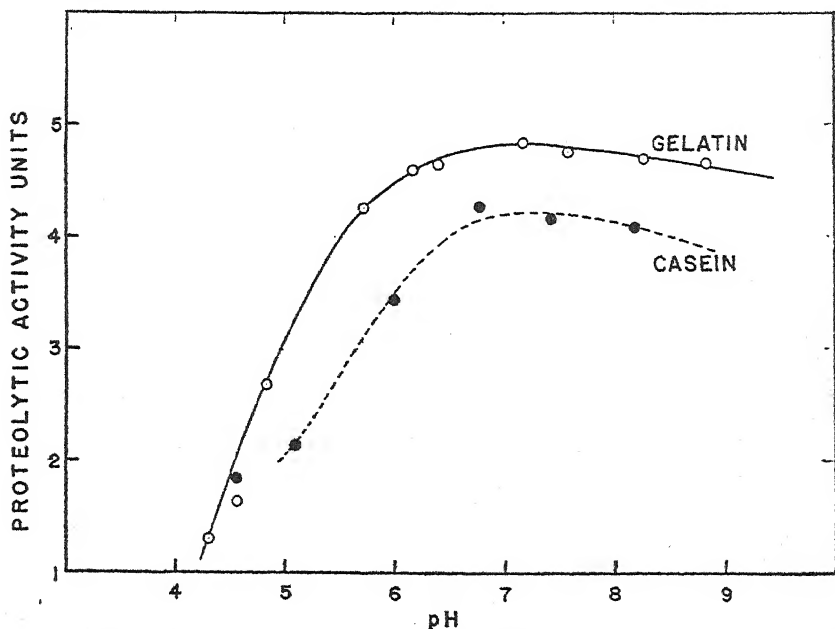


Fig. 2. Effect of pH on the proteolytic activity of a 50% glycerol extract of germinated soybeans. Proteolytic activity units; ml of 0.05N KOH per 10 g soybeans.

In Figure 2 is recorded the soybean proteolytic activity with varying pH values, controlled by adding varying amounts of citrate buffer to the digests, using the substrate gelatin in one set of experiments and casein in the other. With increasing pH, the activity increased rapidly until an optimum was reached between pH 6.5 and pH 7.0. A further increase in pH caused only a slight decrease in activity.

### Summary

Soybean seeds are a good source of beta-amylase. They contain, however, only a trace of alpha-amylase. Germination does not affect these enzymes to any extent. Unlike barley and wheat, soybeans do not contain bound beta-amylase. The optimum pH of the soybean amylase has been found to be in the neighborhood of pH 5.9.

"Soyin," the protease of soybean, increases considerably during germination. It is extracted efficiently with 30% and 50% glycerol solutions. On casein and gelatin substrates, the activity of "Soyin" increases rapidly with increasing pH, until an optimum is reached at approximately pH 6.7-7.0, and decreases slightly in the alkaline range.

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## THE LOSS OF THIAMINE ON COOKING BREAKFAST CEREALS

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The stability of thiamine in natural substances has long been known to depend on temperature (Holst, 1907; Chick and Hume, 1917; Vedder, 1918; Sherman and Grose, 1923; and Keenan, Kline, Elvehjem, and Hart, 1935). That pH also influenced the destruction of the vitamin by heat was demonstrated by Sherman and Burton (1926). Considerable data have been accumulated regarding the effect of these

factors on the loss of thiamine during the preparation of foodstuffs under various conditions.

Destruction of pure thiamine on heating depends on time and pH (Farrer, 1941). Beadle, Greenwood, and Kraybill (1943) studied the effect of temperature and pH on solutions of thiamine and found that the amount of destruction depended not only on time, temperature, and pH, but also on the nature of the electrolyte present. Cocarboxylase, the pyrophosphate of thiamine, was more stable than the vitamin under similar test conditions (Greenwood, Beadle, and Kraybill, 1943).

The experiments reported herein were carried out to investigate the relative stability of thiamine and cocarboxylase, and to determine the cooking loss of the vitamin in cereals fortified with thiamine and cocarboxylase.

### Experimental

The thiochrome method of Hennessy and Cerecedo (1939), as modified by the Research Corporation Committee on the Thiochrome Method (Hennessy, 1941), was used throughout this investigation.

*Destruction of Thiamine and Cocarboxylase in Buffered Solutions.* Five  $\mu$ g of synthetic thiamine hydrochloride and 5- $\mu$ g equivalents of thiamine as cocarboxylase were placed in separate 25 ml volumetric flasks containing 10 ml of a standard buffer solution. MacIlvaine's citric acid-disodium phosphate buffer was used to acquire pH values up to 6.25 and Clark and Lubs' potassium acid phosphate-sodium hydroxide buffer was employed for pH values greater than 6.25. The flasks were autoclaved for 15 min at 120°C, cooled immediately, and neutralized to pH 4.5, using bromocresol green as an outside indicator. One ml of a 10% takadiastase solution was then added to each flask and the volume adjusted to 25 ml. The contents of the flasks were mixed and incubated overnight at 40°C. The same procedure was carried out on control solutions except that autoclaving was omitted.

The results illustrated in Figure 1 demonstrate that cocarboxylase was more labile to heat than thiamine at each pH tested. This cannot be attributed to incomplete splitting of the cocarboxylase by the enzyme, since unheated controls showed practically quantitative recovery.

*The Effect of pH on the Loss of Thiamine and Cocarboxylase During Cooking.* To show a practical application of the above results, two samples of farina were studied. One was fortified with thiamine and the other with cocarboxylase (approximately 4- $\mu$ g equivalents of thiamine per g). Five-g samples of each farina were weighed into 100 ml Kohlrausch flasks and 40 ml of distilled water added. Five

different pH values were obtained by adding disodium phosphate at levels of 0.0, 0.5, 1.0, and 4.0 percent. The samples were placed in a flowing steam cooker (100–101°C) for time intervals of 5, 15, 30, and

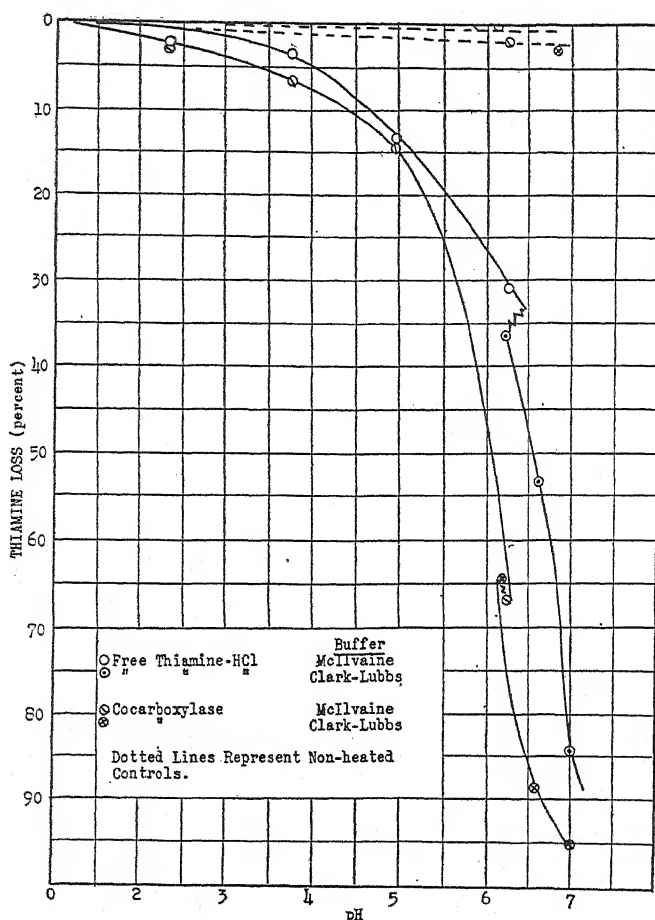


Fig. 1. Comparative stability of cocarboxylase and thiamine hydrochloride to heat at different hydrogen-ion concentrations. Solutions containing 0.5  $\mu$ g equivalents of thiamine hydrochloride were autoclaved for 15 min at 15 lb pressure.

60 min. Control samples were treated with 5 ml of 1N  $H_2SO_4$  before cooking.

After cooking, 5 ml of 1N  $H_2SO_4$  were added to all of the samples except the controls, and the entire series autoclaved at 120°C for 15 min. The samples were cooled and incubated overnight at 40°C with takadiastase. The solutions were neutral to bromocresol green (pH

4.5) before incubation. A Coleman electrometer was used to determine the pH of the cooked samples.

Tables I and II show that the cooking loss increased with pH and time of cooking, and was greater for farina enriched with cocarboxylase than for that enriched with pure thiamine.

TABLE I  
LOSS OF THIAMINE DURING COOKING OF FARINA FORTIFIED WITH PURE THIAMINE HYDROCHLORIDE (100–101°C)

Sample	pH after cooking	Cooking time in minutes							
		5		15		30		60	
		Thia- mine	Loss	Thia- mine	Loss	Thia- mine	Loss	Thia- mine	Loss
		μg/g	%	μg/g	%	μg/g	%	μg/g	%
Control	2.0	4.14		4.14		4.14		4.14	
0.0% Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	5.8	4.17	+0.7	4.05	2.2	3.91	5.56	3.72	10.1
0.5% Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	6.6	4.35	+5.08	3.90	5.8	3.86	6.76	3.32	19.8
1.0% Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	6.9	3.92	5.32	3.64	12.1	3.31	20.0	2.89	30.2
2.0% Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	7.2	3.72	10.1	3.42	17.4	2.66	35.8	2.00	51.7
4.0% Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	7.5	3.56	14.0	3.24	21.8	1.64	60.5	0.74	82.2

TABLE II  
LOSS OF THIAMINE DURING COOKING OF FARINA FORTIFIED WITH PURE COCARBOXYLASE (100–101°C)

Sample	pH after cooking	Cooking time in minutes							
		5		15		30		60	
		Thia- mine	Loss	Thia- mine	Loss	Thia- mine	Loss	Thia- mine	Loss
		μg/g	%	μg/g	%	μg/g	%	μg/g	%
Control	2.0	3.80		3.80		3.80		3.80	
0.0% Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	5.8	3.89	+2.3	3.48	8.42	3.12	17.9	2.90	23.7
0.5% Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	6.6	—	—	—	—	2.77	27.1	2.01	47.1
1.0% Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	6.9	—	—	2.88	24.2	2.14	43.7	1.49	60.8
2.0% Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	7.2	—	—	2.49	34.5	1.57	58.7	0.84	77.9
4.0% Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	7.5	—	—	1.62	57.4	0.76	80.0	0.27	92.8

*Thiamine Loss During Cooking of Some Commercial Breakfast Cereals.* For this study, samples of commercial cracked wheat, oatmeal, enriched farina, and a quick-cooking farina (with increased pH) were employed. The thiamine content of the samples was determined in the usual manner. The samples were then cooked for intervals of 5, 15, 30, 60, and 120 min at 100–101°C in a flowing-steam cooker. After cooking, determinations of pH and of thiamine content were made.

Table III shows that, in general, the vitamin loss during cooking increased with increasing pH values. None of the cereals sustained a significant loss of thiamine for cooking periods of 5 and 15 min. The

TABLE III  
LOSS OF THIAMINE DURING COOKING OF BREAKFAST CEREALS (100–101°C)

Cereal	Thia- mine con- tent	pH after cook- ing	Cooking time in minutes									
			5		15		30		60		120	
			Thia- mine	Loss	Thia- mine	Loss	Thia- mine	Loss	Thia- mine	Loss	Thia- mine	Loss
	μg/g		μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
Quick-cooking farina, enriched	5.42	6.85	5.43	0.0	4.91	9.4	4.46	17.7	4.07	25.0	3.21	40.8
Cracked wheat	4.47	6.30	4.28	4.25	3.89	13.0	3.88	13.2	3.61	19.2	3.28	26.6
Oatmeal	5.74	6.20	5.75	0.0	5.60	2.44	5.38	6.28	5.18	9.77	4.60	19.9
Enriched farina	4.14	5.80	4.17	0.0	4.05	2.2	3.91	5.56	3.72	10.1	3.52	15.0

cracked wheat and quick-cooking farina both showed appreciable losses during the longer cooking times.

### Discussion

Some early investigators reported that heating or cooking of food-stuffs did not destroy thiamine, while other workers presented evidence to show that destruction did occur. Williams (1939) suggested that much of the conflicting evidence was due to differences in the thermostability of the free vitamin and cocarboxylase. The results of the present study indicate that foodstuffs rich in cocarboxylase would show more cooking loss than those rich in the free vitamin. Our data are not in accord with the report by Greenwood, Beadle, and Kraybill (1943) that cocarboxylase was more stable to heat than the free vitamin. However, the test conditions in their experiments were different from those described here; since these workers observed that the stability of pure thiamine at any one pH depended on the buffer system employed, it is possible that cocarboxylase may manifest similar properties.

Although 5 min is sufficient time to cook breakfast cereals such as farina and oatmeal, the time is often extended in many cases. This is especially true in the preparation of cooked cereal for large groups, such as in our armed forces, or in restaurants and institutions where it may be left on a steam table for several hours. Prolonged cooking is undesirable since there is a significant loss of thiamine, especially in cereals with a high pH.

### Summary

Pure cocarboxylase was more readily destroyed by heat than thiamine in buffered solutions at pH values of 3.5 to 7.0.

The vitamin loss during cooking, at pH values between 5.8 and 7.5, was greater in the case of farina fortified with cocarboxylase than when thiamine was used.

Cooking tests with enriched farina, oatmeal, cracked wheat, and quick-cooking enriched farina showed that the thiamine loss was negligible for short cooking periods. The loss was increased when the cooking time was prolonged, especially in the instance of cracked wheat and farina which were cooked at pH levels of 6.3 and 6.8 respectively.

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# THIAMINE RETENTION IN THE COMMERCIAL PRODUCTION OF ZWIEBACK

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There is considerable interest in the extent of loss of thiamine in bakery products during processing. The Baking Industry Advisory Committee of the War Food Administration has recommended the extension of the enrichment program to baked goods other than bread which are capable of conveying to the consumer a full measure of the vitamins added. Factual data on the bake-out loss of thiamine in the production of many of the sweet, yeast-raised products are lacking. The present report provides some data on the retention of thiamine in the commercial preparation of Zwieback, a product which is subjected to both baking and toasting operations.

## Materials and Methods

A commercial dough of the customary size was enriched by the use of tablets<sup>1</sup> in a commercial bakery. The dough formula which was selected and other pertinent information are provided in Table I. The

TABLE I  
DOUGH FORMULA SELECTED FOR ZWIEBACK

Ingredient	%	lb	oz
Flour	100	300	—
Water	53	159	—
Yeast	2	6	—
Salt	0.8	2	4
Sugar	14	42	—
Dried skim milk	1	3	—
Malt	2	6	—
Shortening	4	12	—
Flavoring materials	0.11	—	5.5
Vitamins and iron	tablets	—	—

Mixing time—7 min (high speed).

Dough temperature out of mixer—26.1°C (79°F).

Total fermentation time—3 hr at 26.7°C (80°F); (punched at 2½ hours).

dough was divided into 14-oz (400 g) loaves by a Baker Perkins six-pocket divider.

Considerable pains were taken to secure representative samples for thiamine assay of the dough, of the bread made therefrom, and of the

<sup>1</sup> The product known as B-E-T-S of the Winthrop Chemical Co. was used.

finished Zwieback. Four loaves, one from each quarter of the dough, were accurately weighed (400 g) and panned. These loaves were removed from the pan for assay after proofing, as the rest of the dough went to the oven. The loaves were flattened upon one another and a cross section cut for assay.

In a like manner, 16 other loaves, one loaf taken from each third cut as the dough passed through the divider, were weighed, panned in four four-strap pans, and proofed. A Baker Perkins tunnel type oven which was used for baking and toasting accommodates five of these pans from side to side of the oven. To obtain loaves baked at both center and side of the oven, the 12 loaves contained in the three pans from the center to one side of the oven were selected for slicing and toasting. The remaining pan, on the opposite side between center and side pan, was used for the assay of the untoasted loaves. The oven temperatures and time of baking and toasting are given in Table II.

TABLE II  
OVEN TEMPERATURE AND TIME IN PRODUCTION OF ZWIEBACK  
(Tunnel type oven)

Operation	Temperature								Total time in oven
	First section		Second section		Third section		Fourth section		
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	
Baking	°C 168	°C 204	°C 141	°C 174	°C 177	°C 154	°C 160	°C 168	<i>min</i> 27
Toasting	174	188	132	146	177	152	154	166	19

After cooling and drying about 24 hours on the rack, the 12 loaves were put through the slicing machine and cut into  $\frac{5}{8}$ -inch slices. Weighings were made before and after slicing to record the weight of crumbs lost in slicing. The slices were laid as close together as possible on rectangular heavy metal screens for toasting; five screens just filled the space from side to side on the floor of the oven. The 12 loaves covered more than three screens, giving a representative sample from side to center of the oven. All screens were covered with slices and the oven was full throughout the toasting.

The Zwieback obtained from the entire 12 loaves was ground and thoroughly mixed for a sample. Likewise the four loaves set aside for bread analysis were sliced, air-dried, ground, and thoroughly mixed.

Thiamine was determined by the collaborative thiochrome method of Hennessy (1942), as outlined in Cereal Chem. Bull. 2 (2): 25-29, and

the results expressed in terms of the total thiamine present in four loaves in the form of dough, bread, and Zwieback respectively.

### Results and Discussion

It will be noted from the results in Table III that the percentage of the total thiamine of the dough lost in toasting the sliced loaves (20%),

TABLE III  
MEAN THIAMINE LOSSES IN BAKING AND TOASTING

Milligrams of thiamine in four loaves			Percent of total thiamine of dough lost		
Dough	Bread	Zwieback	In baking	In toasting	Total loss
4.86	3.99	3.02	18	20	38

is not much greater than the percentage lost in baking the loaves (18%). This is probably due to the comparatively long, flat shape of the loaves, which results in a low ratio of crumb to outer surface of loaf. Schultz, Atkin, and Frey (1942) have shown that thiamine destruction in baking is much greater in the crust than in the crumb of the loaf of bread.

If the toasting loss is calculated in terms of the percentage of the total thiamine of the bread instead of the dough, we find the toasting loss to be 24%. The relatively high sugar content of this product results in obtaining the desired color without the application of unduly high temperatures or long toasting times, either of which would result in greater destruction of thiamine. Hoffman, Schweitzer, and Dalby (1940) found a loss of 26% in the preparation of Melba toast from high B<sub>1</sub> white bread. Melnick, Mabardie, Bernstein, and Oser (1941) reported a 15% destruction of thiamine in preparing "usual toast." Downs and Meckel (1943) found a toasting loss of 13% of the total thiamine of enriched bread in 50 sec toasting at the temperature of the household toaster. This was the degree of toasting preferred by a majority of persons asked to view the toast samples.

### Summary

A representative Zwieback dough was enriched with commercial enrichment tablets, and the dough, bread, and toast were assayed for thiamine by the thiochrome method. The over-all loss of thiamine was 38%, attributable almost equally to the baking and toasting operations, under the conditions of the experiment.

The toasting loss was 24% when calculated on the total thiamine of the bread.

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## A PREHARVEST SURVEY OF THE PROTEIN CONTENT OF WESTERN CANADIAN WHEAT OF THE 1943 CROP<sup>1</sup>

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(Manuscript received, April 13, 1944; presented at the Annual Meeting, May 1944)

Since 1927 the Board of Grain Commissioners' Laboratory has made annual surveys of the protein content of Western Canadian hard red spring wheat, and data for the first 12 of these have been summarized by Anderson and Eva (1943). Because the annual survey cannot be completed until samples have been obtained from northern areas where the harvest is late, and because additional time is required for mapping, tabulating, typing, and printing, it has rarely been possible to distribute the survey report before mid-November. Some information has been made available earlier by issuing preliminary and interim reports to the press, but these cannot contain detailed data. In one year when conditions were advantageous the final report was distributed on October 29, but in some years it has been delayed until December. Moreover, for the past three years, transport and storage problems have made it necessary to place certain restrictions on the delivery of wheat, especially during the early part of the crop year, and this has made it increasingly difficult to obtain samples for an early protein survey. In these circumstances it seemed desirable to investigate the possibilities of a preharvest survey. An initial study was made in 1943 and is reported in this paper.

Other similar studies have dealt with preharvest surveys of yield, and King and Jebe (1940) have reviewed the literature and contributed a valuable study of sampling problems. In their survey, a two-man team covered each crop-reporting district by car, following three evenly

<sup>1</sup> Paper No. 65 of the Grain Research Laboratory, Winnipeg, and No. 223 of the Associate Committee on Grain Research (Canada).

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spaced routes running north and south and three running east and west. One field was sampled after every two miles of wheat frontage, and a procedure for ensuring random sampling within fields was followed. The investigation showed that this method of estimating and forecasting yield per acre was both practical and efficient.

For our study, a systematic attempt to approach the ideal of random sampling did not seem feasible. The area to be covered was too large, and with current shortages of manpower, gasoline, and tires, the use of a car was impossible. The investigation was therefore designed to determine whether the simplest practical method of making a preharvest survey would give results comparable with those obtained in the postharvest survey. For the postharvest surveys, it is the practice to collect 5,000 or 6,000 samples of farmers' deliveries to elevators in such a way that numbers of samples are roughly proportional to production in each district. But for the preharvest survey, only about 1,500 samples—taken by country elevator agents from wheat fields just prior to harvesting—were collected, and no attempt was made to obtain proportionality between sampling and production. In both surveys, only a general picture of the distribution of zones of different protein levels was sought; indeed, this is all that is required for practical purposes. For this reason it was not necessary to design the investigation with detailed statistical analyses of the data in mind. It was thought that maps would serve best to summarize and present the results in such a way that the reader could check the writers' conclusions. A liberal use of maps has therefore been made.

## Materials and Methods

*Preharvest Samples.* The samples for the preharvest survey were collected by asking 3,316 of the agents at country elevators in Western Canada to collect one sample apiece in accordance with the instructions given below. It will be apparent that the task of taking samples was made as simple as possible; a deliberate attempt was thus made to facilitate the cooperation of busy agents.

*Who Takes Samples?* Each buyer of a Line Elevator Company is asked to obtain one sample.

*Selection of Wheat Field.* If you are the only Line Elevator buyer at point you may take the sample from any conveniently located field of wheat. If there are two or more Line Elevator buyers at your point, it will be necessary for you to get together and arrange to take samples from different farms.

*Time for Taking Sample.* The sample shall be taken as soon as the field is ripe and before it is cut.

*Method of Taking Sample.* It is left to your good judgment to take an average sample of the field. Avoid the early spots on high ground, and the late spots in hollows.

*Size of Sample.* The investigation requires a sample of about 4 ounces (130 grams). Collect enough heads to yield this amount.

*Threshing of Sample.* If the sample is not dry enough for immediate hand threshing, hang it up in your office for a day or two. When the heads are dry, put them in a small cloth bag and beat them with a stick. Empty the bag, blow off the chaff, and enclose the grain in a sample envelope on which appears your company name and shipping point.

*Mailing of Sample.* Stick the enclosed label to sample envelope, and mail.

A total of 1,620 samples was received (49% of requests), but 116 of these were spoiled because they were not dried sufficiently before shipment; 1,504 samples were therefore available for analysis.

*Postharvest Samples.* In the early part of the crop year it is the practice for elevator agents at all points to send many envelope samples of farmers' deliveries of new wheat to the head office of their companies. This procedure makes it possible to check the uniformity of buying operations at all points. Through the kindness of the companies these samples are made available to the Grain Research Laboratory. From them there is selected a series of samples of the contract grades which provides good coverage of all wheat-growing areas. By making the number of samples taken from each crop district proportional to wheat production in the district, the survey is "weighted" to give a close estimate of the mean protein content of the crop. This method is the best of the procedures that have been used by this laboratory. For further information on methods, the reader is referred to the bulletin by Anderson and Eva (1943) dealing with protein surveys of Western Canadian wheat for the 12 years 1927 to 1938.

*Analytical Methods.* It was necessary to air-dry some of the pre-harvest samples in the laboratory. All samples were cleaned in an Emerson Kicker, and moisture was determined with a motor-driven Tag-Heppenstall moisture meter calibrated to give the same results as the Brown-Duvel method. Nitrogen determinations were made by the Kjeldahl-Gunning-Arnold method on a 1-g sample of ground wheat (Hobart coffee mill) and protein data ( $N \times 5.7$ ) are reported on a 13.5% moisture basis.

*Preparation of Maps.* Two types of maps, dot and hatched, are presented. Separate dot maps are used for different protein ranges, and one dot is shown for each shipping point having a mean protein level within the specified range. Hatched maps are also based on the mean protein levels for shipping points. The mean data are transferred to a 12 X 20 inch base map on which a different colored dot is used for each 1.0% range in protein content. As the mean protein levels tend to change relatively uniformly from one district to another, it is not difficult to draw zone boundaries separating dots of different colors. When one or two shipping points have protein levels outside the range for surrounding points, the area is frequently too small to show as a separate zone; but when two or three such points are fairly

close together it is generally possible to outline a zone that is large enough to reproduce on a small-scale map. Zone boundaries are subsequently transferred to a 6 X 10 inch base map, the zones are hatched with "Zip-A-Tone," and the map is photographed.

### Results and Discussion

It is important to deal with the relative dates by which pre- and postharvest surveys were completed, and this point is discussed first. The accuracy with which the preharvest survey predicted the geographic distribution of zones of different protein level—as determined in the postharvest survey—is then considered with the aid of dot and zone maps for both surveys. And in order to provide perspective, the preharvest zone map is also compared with zone maps for each of the past 16 years. Mean protein levels for crop districts, correlation and regression coefficients for pre- and postharvest means, and the accuracy with which the mean protein levels for the whole crop were predicted, are then discussed.

*Comparative Rates of Collection of Samples.* The rates at which samples were received are illustrated in Figure 1. By October 1,

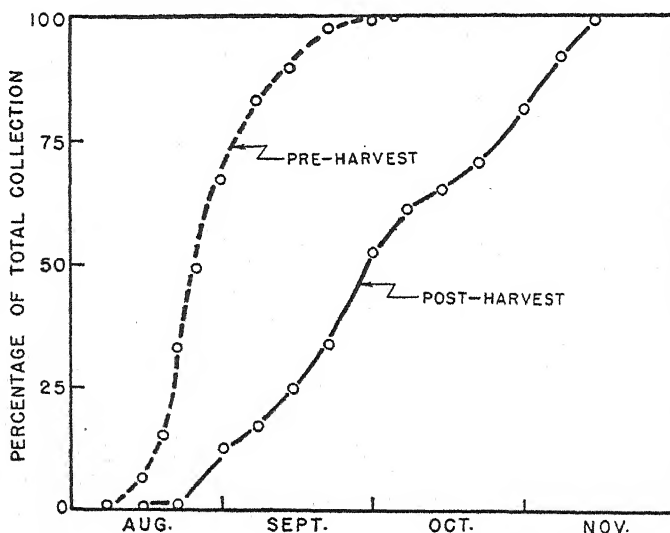


Fig. 1. Curves showing the rates at which preharvest and postharvest samples were received.

almost all of the 1,620 samples for the preharvest survey had been received, but only 52% of the 5,645 postharvest samples had been collected. In both surveys the principal difficulty is that of obtaining samples from northern areas where the harvest is late. For instance, almost all of the preharvest samples received after September 15 came

from north-central Alberta and the Peace River Block. Thus in 1943 it would have been impossible to cover the whole crop-growing area adequately before October 1 unless the northern samples had been taken before the grain was ripe. In spite of this limitation, the pre-harvest survey was completed over a month before the postharvest survey. This represents an appreciable saving of time; and in many years, when the harvest is reasonably early, it should be possible to complete the preharvest survey two weeks earlier. When the harvest is later in the North, it may well prove practical to take the samples on September 10, and to make special arrangements for drying and shipping them, so that the survey can be completed by mid-September.

Preliminary maps covering southern and eastern areas where the harvest is early can be prepared several weeks earlier for both surveys. This practice has been followed for some years with the postharvest surveys. In the present study, however, the object was to compare procedures for obtaining reasonably accurate maps for the whole of the crop-growing area. Accordingly, it does not seem advisable to discuss preliminary maps.

*Dot Maps for Pre- and Postharvest Surveys.* A pair of maps representing pre- and postharvest data are shown in Figure 2 for each of four protein ranges: under 11.0%, 11.0 to 12.9%, 13.0 to 14.9%, and over 14.9%. In each map, one dot was entered to represent each shipping point that had a mean protein level within the range for the map. The number of shipping points represented on each map, and the number of points common to both maps, are shown in Table I.

TABLE I  
NUMBER OF SHIPPING POINTS REPRESENTED IN EACH SURVEY

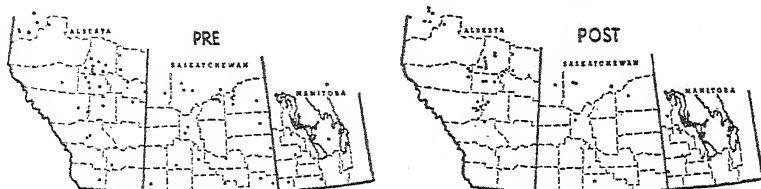
Protein range for maps	Number of shipping points		
	Preharvest map	Postharvest map	Common to both maps
Under 11.0%	63	49	9
11.0 to 12.9%	247	392	102
13.0 to 14.9%	494	836	282
Over 14.9%	224	124	50
Total	1,028	1,401	

It is not entirely fortuitous—though unpremeditated—that so few of the shipping points are common to both surveys; four large companies which supplied the majority of the postharvest samples were not asked to collect preharvest samples. This disparity between surveys creates no special difficulty in interpreting the data by means of maps,

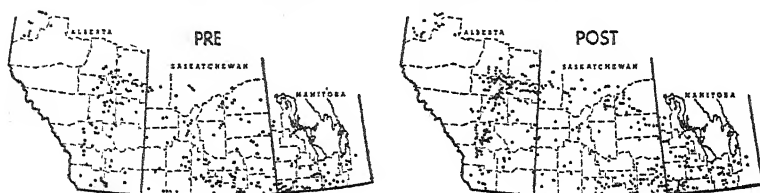


but may have caused slightly greater variation in the mean values for crop districts (discussed in a later section) than would have occurred if the same set of points were represented in both surveys.

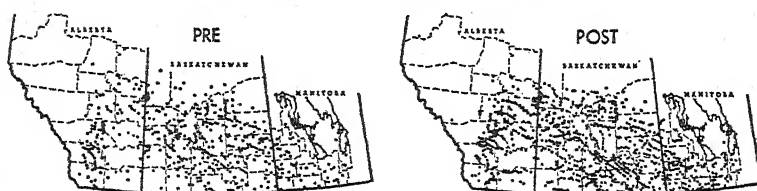
(A) PROTEIN CONTENT UNDER 11.0%



(B) PROTEIN CONTENT, 11.0 – 12.9%



(C) PROTEIN CONTENT, 13.0 – 14.9%



(D) PROTEIN CONTENT OVER 14.9%

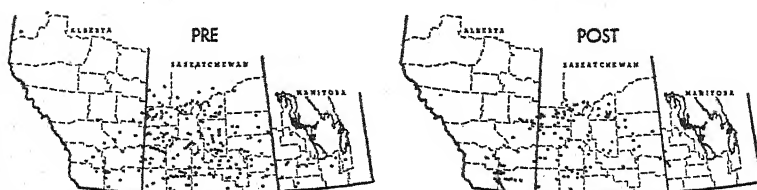


Fig. 2. Maps of Western Canada showing the distribution of shipping points from which preharvest and postharvest samples of different protein ranges were received.

The dot maps represent the most extensive and detailed treatment of the raw data presented in this paper. Nevertheless they can be allowed to speak for themselves. The reader will doubtless agree that there is great similarity between the geographic distributions of dots

within each pair of maps. But when the distribution of dots between preharvest maps is compared with the distribution between postharvest maps, considerable differences are apparent. These result largely because the number of samples taken from different areas was made roughly proportional to production in the postharvest survey, whereas it was not consistent with the required simplicity to "weight" the preharvest collections in this manner; accordingly, the areas of heavy production, in which protein levels of 13.0 to 14.9% predominate, are

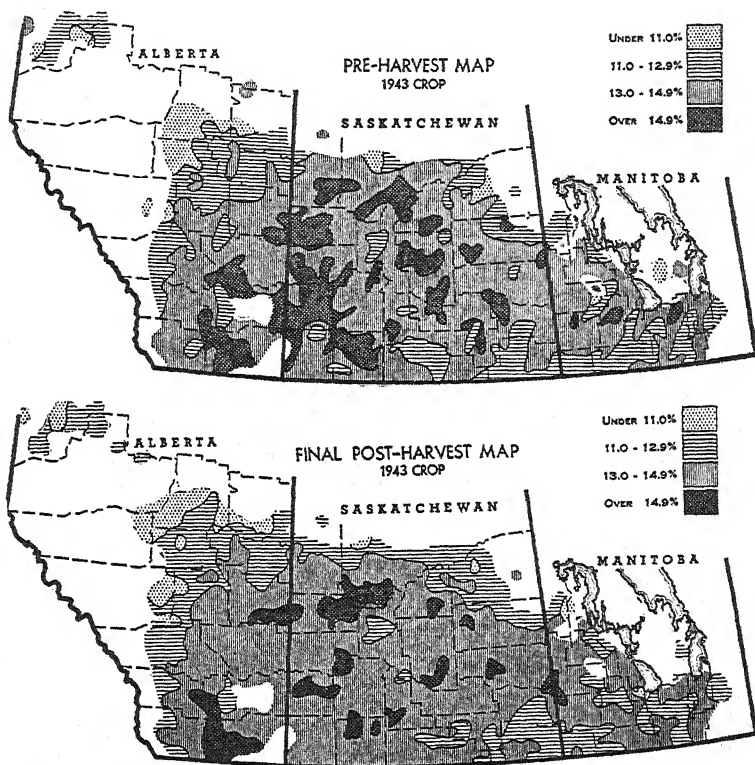


Fig. 3. Maps of Western Canada showing protein zones outlined from the data for the preharvest survey (top), and from the data for the postharvest survey (bottom).

better represented in the postharvest survey. However, this difference in method does not seem adequate to explain why the preharvest map for the highest protein range shows considerably more shipping points in central and southern Saskatchewan, and in southeastern Alberta, than does the postharvest map. It seems probable that the preharvest survey overestimated protein levels in parts of these areas. The writers have not been able to find an adequate and convincing explanation of this difference between the two surveys. It may well be related

to variations in the weather at harvest times in different districts; but the area covered by the surveys is so large, and the detailed information on weather is so meager, that investigation of this matter does not appear feasible.

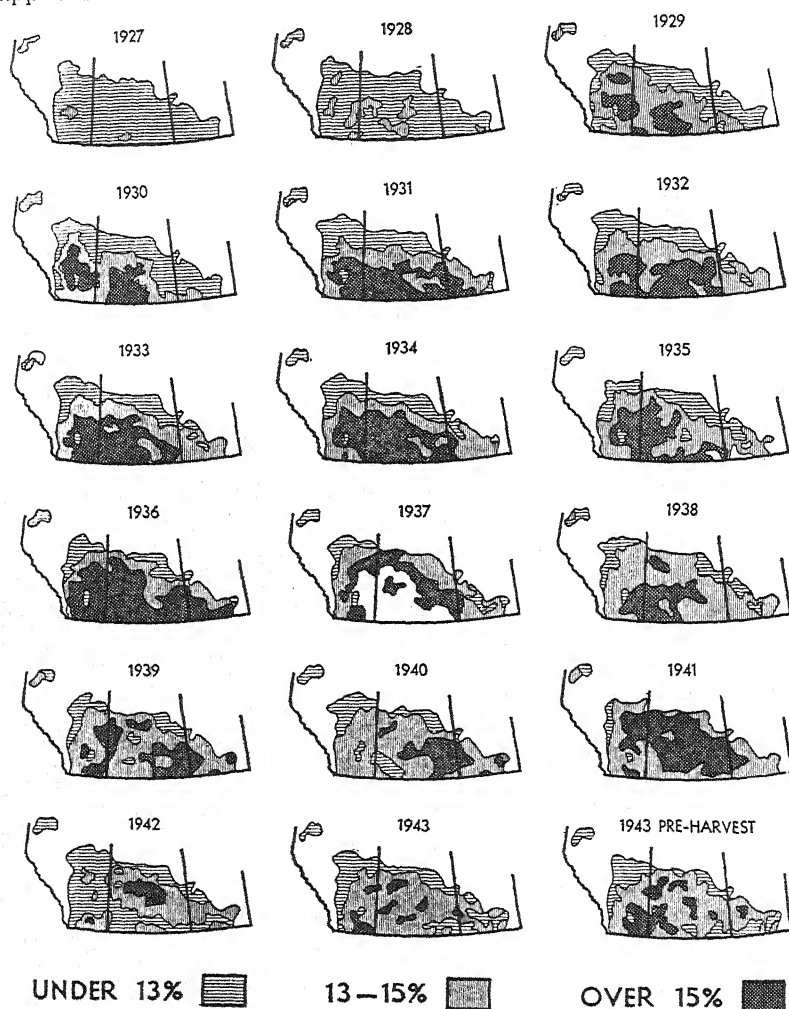


Fig. 4. Protein maps of Western Canada for the postharvest surveys of the years 1927 to 1943, and for the preharvest survey of 1943.

*Hatched Maps for Pre- and Postharvest Surveys.* The data presented in Figure 2 are summarized in the pair of hatched maps that appear in Figure 3. The preharvest map (top) shows larger zones of high protein level (dark crosshatching) than the postharvest map (bottom), especially throughout eastern Saskatchewan. Though the agreement for

other zones is by no means perfect, the writers believe that it is adequate for practical purposes and better than might have been expected in view of the methods used for the two surveys.

*Comparison of 1943 and Earlier Maps.* The usefulness of the preharvest map for 1943 can be determined—and put in perspective—by comparing it not only with the postharvest map for 1943 but also with those for as many other years as possible. Accordingly, maps for the years 1927 to 1942, and the two maps for 1943, are shown in Figure 4. As these maps are reproduced on a small scale, only one range is shown below 13.0% protein, and the zones are outlined in less detail; some of the smaller ones are left out, and others have been joined to make a single, larger area.

It will be readily agreed that the preharvest map for 1943 resembles the postharvest map for 1943 much more closely than it resembles the postharvest map for any of the other 16 years. The writers believe that this in itself is enough to establish the preharvest survey—carried out by the simple means used in this investigation—as a promising procedure for obtaining early information on each crop.

*Mean Protein Levels.* In order to make a reasonable comparison of the pre- and postharvest data in terms of mean protein levels, it is necessary to discount the normal variation—frequently in units of as much as 5%—that occurs between samples collected from one shipping point. This can be done by working with areas that are large enough to be represented by a considerable number of samples; and for this purpose the crop districts (Fig. 5) are a logical choice.

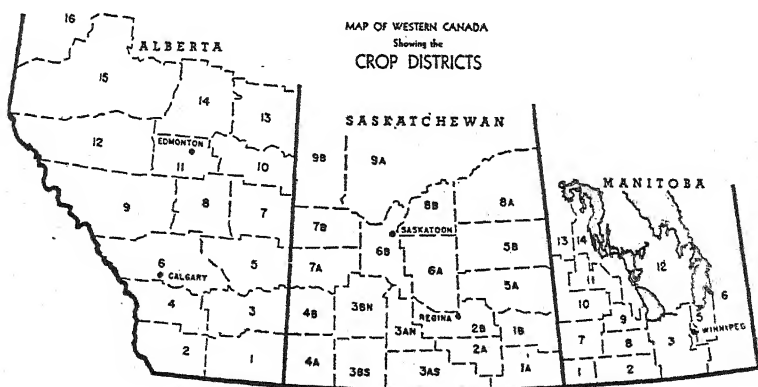


Fig. 5. Map of Western Canada showing crop districts and principal towns.

The mean protein levels and the number of samples for each crop district, for both pre- and postharvest surveys, together with data on wheat production, are given in Table II. Agreement between the pre-

TABLE II  
PROTEIN LEVELS, NUMBERS OF SAMPLES, AND PRODUCTION FOR CROP DISTRICTS

Province	Crop district	Protein content		Number of samples		Production  1,000 bu.
		Pre-harvest	Post-harvest	Pre-harvest	Post-harvest	
		%	%			
Saskatchewan	4B	15.2	14.6	52	76	2963
Alberta	1	15.0	15.0	23	125	5239
Saskatchewan	7B	15.0	14.7	40	112	3661
Saskatchewan	3BS	15.0	14.2	60	140	9414
Saskatchewan	8B	14.5	14.2	49	214	8314
Saskatchewan	1B	14.5	13.4	17	119	6765
Saskatchewan	7A	14.4	14.3	68	145	5477
Saskatchewan	3BN	14.4	14.1	70	152	7834
Saskatchewan	6A	14.4	14.1	74	177	10680
Saskatchewan	2B	14.2	14.1	66	209	13797
Alberta	3	14.2	13.9	20	76	3451
Alberta	5	14.1	14.0	22	122	3655
Saskatchewan	5B	14.1	13.7	64	203	8837
Saskatchewan	3AN	14.0	13.8	35	129	5573
Saskatchewan	9B	14.0	13.5	26	143	6164
Saskatchewan	9A	13.9	13.9	41	164	9530
Manitoba	7	13.9	13.4	22	112	4627
Saskatchewan	5A	13.8	13.8	57	185	13073
Saskatchewan	6B	13.8	13.8	63	212	10543
Alberta	4	13.8	13.7	59	194	7716
Saskatchewan	2A	13.8	13.4	34	107	7979
Manitoba	9	13.7	13.3	3	38	1756
Alberta	7	13.5	13.7	31	162	8536
Manitoba	11	13.5	13.1	4	50	2364
Saskatchewan	4A	13.4	13.9	23	43	2265
Alberta	2	13.4	13.3	30	114	4513
Manitoba	10	13.4	13.0	16	95	4576
Saskatchewan	3AS	13.3	13.2	53	206	15438
Alberta	6	13.2	13.3	46	274	13668
Manitoba	3	13.2	13.1	39	177	8793
Saskatchewan	1A	13.2	12.7	28	149	8557
Manitoba	8	13.1	13.2	22	72	3315
Manitoba	2	12.8	13.1	22	140	6835
Saskatchewan	8A	12.6	12.6	41	112	5219
Alberta	8 and 9 <sup>1</sup>	12.4	12.6	33	182	8356
Manitoba	4, 5, and 6 <sup>1</sup>	12.3	12.6	9	36	2177
Alberta	10	12.3	12.6	47	256	11224
Manitoba	1	12.1	12.9	19	74	3931
Manitoba	12, 13, and 14 <sup>1</sup>	12.1	12.8	8	35	2018
Alberta	15 and 16 <sup>1</sup>	12.0	11.6	25	77	3901
Alberta	13	11.6	11.5	6	45	2600
Alberta	11	11.1	12.0	11	83	3277
Alberta	14	10.8	11.0	17	103	7554
Alberta	12	—	10.2	—	6	456

<sup>1</sup> Crop districts in which little wheat was grown, and from which few samples were obtained, were grouped with adjacent districts.

and postharvest means is reasonably good: 60% of the crop districts, including most of those in which production was high, yield means differing by 0.3% or less; and for an additional 26%, the difference is not over 0.5%.

It is also interesting to compare the pre- and postharvest means for the provinces and for Western Canada as a whole. This can be done in three ways: by comparing the unweighted means (of the crop district means); by comparing means weighted in accordance with the number of samples for each crop district (actually the means for all samples); and by comparing means weighted for production in each crop district. The three sets of data are shown in Table III.

TABLE III  
MEAN PROTEIN LEVELS FOR CROP DISTRICTS FOR EACH SURVEY

Province	Survey	Mean protein levels		
		Of crop district means	Weighted by number of samples	Weighted by production
Manitoba	Preharvest	13.0	13.0	13.1
	Postharvest	13.0	13.1	13.1
Saskatchewan	Preharvest	14.1	14.1	14.0
	Postharvest	13.8	13.8	13.8
Alberta	Preharvest	12.9	13.1	12.9
	Postharvest	12.9	13.1	13.0
Western Canada	Preharvest	13.46	13.75	13.55
	Postharvest	13.36	13.47	13.43

The three methods of calculating the means give much the same results. All three methods show good agreement for Alberta and Manitoba between pre- and postharvest means, but it is apparent that the preharvest survey overestimated the protein level for Saskatchewan. The agreement for Western Canada as a whole is very good; the means weighted by production, the best method of obtaining an accurate estimate, show a difference of only 0.12% between pre- and postharvest values. As there may well be some doubt as to whether the postharvest survey is accurate to  $\pm 0.1\%$ , or even to  $\pm 0.3\%$ , the estimate provided by the preharvest survey must be considered eminently satisfactory.

*Correlation Studies.* The correlation between the pre- and postharvest protein levels for crop district means is  $r = 0.93$  ( $n = 41$ ). The scatter diagram is shown in Figure 6. Different types of dots have been used for the data for each province, and it is apparent that the swarms are relatively homogeneous.

The regression coefficient is 0.75. Under ideal conditions, and with a correlation of 1.0, a regression coefficient of 1.0 might also be obtained. As it is a property of the regression coefficient to decrease when the correlation coefficient falls as a result of extraneous random variations,

the writers are not inclined to stress the fact that the regression coefficient is appreciably lower than 1.0. Although the regression line suggests that the preharvest survey tends to underestimate the low protein values and to overestimate the high protein values, it appears wise to hold this hypothesis in abeyance pending the results of additional studies.

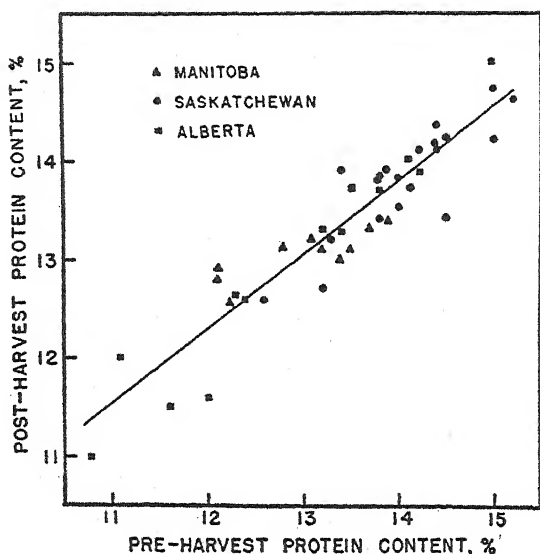


Fig. 6. Scatter diagrams illustrating the correlation between preharvest and postharvest data for the mean protein levels of crop districts in Western Canada.

As a matter of interest, the equation for predicting postharvest values from preharvest data was calculated. It is

$$y = 0.754x + 3.2 \quad (\pm 0.3)$$

where  $y$  is the postharvest protein level and  $x$  is the preharvest value. The standard error of estimate for this equation is  $\pm 0.3$ ; and this appears to be a very useful level of precision.

### Summary

A preharvest survey of the protein content of the 1943 crop of Western Canadian wheat was made with 1,504 samples collected just before harvest by country elevator agents. The results were compared with those given by a postharvest survey made with 5,645 samples taken from grain delivered by farmers to country elevators.

The preharvest protein map resembled the postharvest map much more closely than it resembled corresponding postharvest maps for

any of the preceding 16 years. A correlation coefficient of 0.93 ( $n = 41$ ) was obtained between the pre- and postharvest data for the mean protein levels of crop districts; 86% of the pre- and postharvest means agreed within 0.5%. For the protein content of the whole crop, the preharvest data gave 13.55% and the postharvest data gave 13.43%. The results of the investigations are considered eminently satisfactory, and perennial preharvest surveys are planned.

#### Acknowledgments

The authors are indebted to the North-West Line Elevators Association, Winnipeg, Canada, and especially to those of their elevator agents who collected samples, for their valuable cooperation in this investigation. Thanks are also given to Mr. V. G. Martin, Laboratory Assistant, Grain Research Laboratory, for preparing the figures.

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## EFFECT OF BRAN ON BREAD BAKING

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Recent emphasis on the production of more nutritious bread has aroused new interest in conserving for human consumption the food values of wheat. That whole wheat bread as generally produced is vastly inferior technically to white bread from the viewpoint of the consumer is a well-established fact. Reasons for this inferiority are not completely known. This study was undertaken to find some of the reasons and, if possible, ways of alleviating them in order that better bread might be produced from whole wheat or long extraction flours.

Since white flour differs largely from whole wheat in that it contains only small amounts of bran and germ, one or both of these fractions may be responsible for the undesirable characteristics of whole wheat flour. A logical approach to the problem seemed to be that of superimposing these two constituents separately upon white flour and thus studying their effects.

A review of the literature shows that a number of investigations have been undertaken concerning wheat germ (Sullivan, Near, and

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Foley, 1936; Sullivan, Howe, and Schmalz, 1936 and 1937; Bull, 1937; and Grewe and LeClerc, 1943) but that much less work has been reported on the effect of wheat bran. However, Willard and Swanson (1911) have recorded the influence on baking of bran and bran extracts, as well as of a number of other milling by-products. Similar results on bran extracts are related by White (1913).

### Experimental

*Effect of Granulation of Bran.* It is a rather general opinion that bran has a mechanical effect on the process of baking, serving merely to dilute the gluten and disrupt the gluten films. If this postulation is correct, grinding the bran finer should increase the loaf volume. It was decided to investigate this property. Preliminary work in which bran was ground to different granulations in a Bantam Mikropulverizer without making any attempt to remove the adhering endosperm indicated that a maximum loaf volume would be produced at an intermediate granulation. Since a possible explanation might be that this fine grinding injures the starch granule, as reported by Jones (1940), the following procedure was used. Three different commercial samples were freed from adhering endosperm by passing through the Mikropulverizer with screen removed, followed by sifting through a No. 40 wire on a Great Western experimental sifter. The throughs of this operation were discarded and the overs divided into five portions. These portions were sifted and reground when necessary to obtain the following samples:

- 1—overs 20W
- 2—throughs 20W, overs 40W
- 3—throughs 40W, overs 60 GG
- 4—throughs 60 GG, overs 10XX
- 5—throughs 10XX

Protein, ash, moisture, diastatic activity, proteolytic activity, and crude fiber were determined on these samples, as described in Cereal Laboratory Methods (4th ed., 1941). Cellulose was determined by a method similar to that of Crampton and Maynard (1938). Average diameters of the brans were determined by measuring a large number of bran particles, selected at random, under a microscope containing a scale in the eye-piece. This scale was previously calibrated with a slide micrometer.

These brans were then superimposed on three different commercial bread flours at the rate of 15% (15% moisture basis) and baked in duplicate, using a procedure described in Cereal Laboratory Methods

(4th ed., 1941) and a formula comprised of 100 g flour, 3 g yeast, 5 g sugar, 1.5 g salt, 3 g shortening, and 4 g dry milk solids. In all cases a control loaf was baked each day for comparison with the bran loaves. Since it was found that doughs containing bran were very sensitive to incorrect absorption, the combinations were baked a number of times at different levels of absorption and the maximum volume found for each combination. Results of this work are recorded in Table I. Representative photographs are shown in Figure 1.

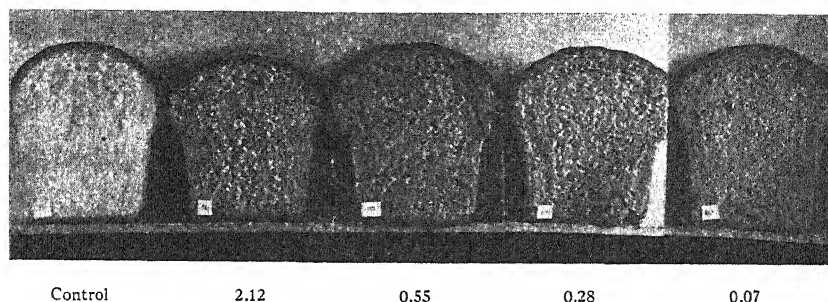


Fig. 1. Loaves of bread baked with various granulations of unwashed bran. Number under each loaf indicates average diameter of particle in mm.

In analyzing these data, the control loaf volumes were subtracted from the corresponding bran loaf volumes and 100 added to the resulting figure. These positive figures were then treated to an analysis of variance, using the second order interaction, brans  $\times$  flours  $\times$  granulations, as the estimate of error. The results of the variance analysis are shown at the foot of Table I.

Correlation coefficients between these figures, which express the response in loaf volume to the addition of bran, and the average diameter of the bran particles are as follows:

Flour series	Correlation coefficient
1	-0.678
2	-0.874
3	-0.844

These correlations are all significant (1% point = 0.641).

*Effect of Bran Extracts.* The large volume of the loaves containing finely ground bran indicates that bran must contain an ameliorating factor which increases loaf volume as well as a destructive factor which influences baking. Accordingly, water, ether, and alcoholic extracts of bran were made. The ether and alcohol were removed from their corresponding extracts by vacuum distillation and the residue added to doughs made from white flour, using the same formula as described before.

TABLE I  
EFFECT OF BRAN PARTICLE SIZE ON LOAF VOLUME

Combination		Size bran particles	Absorption of bran loaf 15% m. b.	Loaf volume		
Flour	Bran			Bran	Control	Response (Diff. + 100)
		mm	%	cc	cc	cc
1	1-1	2.12	67.5	702	720	82
1	2-1	2.35	67.5	738	718	120
1	3-1	2.66	67.5	677	718	59
2	1-1	2.12	69.0	730	792	38
2	2-1	2.35	69.0	788	815	73
2	3-1	2.66	69.0	730	792	38
3	1-1	2.12	70.5	765	800	65
3	2-1	2.35	69.0	712	787	25
3	3-1	2.66	69.0	705	770	35
Average			68.7	727	768	59
1	1-2	1.08	67.5	737	725	112
1	2-2	0.97	69.0	720	730	90
1	3-2	1.16	68.0	725	718	107
2	1-2	1.08	69.0	785	815	70
2	2-2	0.97	69.0	832	827	105
2	3-2	1.16	69.0	770	800	70
3	1-2	1.08	70.5	808	800	108
3	2-2	0.97	69.0	770	787	83
3	3-2	1.16	69.0	747	770	77
Average			68.9	766	775	91
1	1-3	0.55	70.0	772	725	147
1	2-3	0.58	69.0	745	740	105
1	3-3	0.62	70.0	790	725	165
2	1-3	0.55	72.5	780	800	80
2	2-3	0.58	69.0	823	827	96
2	3-3	0.62	71.0	815	815	100
3	1-3	0.55	71.5	772	787	85
3	2-3	0.58	71.0	780	780	100
3	3-3	0.62	71.0	760	782	78
Average			70.6	781	776	105
1	1-4	0.28	71.0	770	720	150
1	2-4	0.33	69.0	790	730	160
1	3-4	0.30	71.0	720	710	110
2	1-4	0.28	74.0	815	815	100
2	2-4	0.33	73.0	810	800	110
2	3-4	0.30	74.5	830	815	115
3	1-4	0.28	72.5	783	780	103
3	2-4	0.33	71.5	805	787	118
3	3-4	0.30	72.0	795	800	95
Average			72.1	791	773	118

TABLE I—*Continued*

Combination		Size bran particles	Absorption of bran loaf 15% m. b.	Loaf volume		
Flour	Bran			Bran	Control	Response (Diff. + 100)
		<i>mm</i>	<i>%</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>
1	1-5	0.07	70.5	802	725	177
1	2-5	0.08	71.5	750	725	125
1	3-5	0.06	71.0	743	718	125
2	1-5	0.07	73.0	833	800	133
2	2-5	0.08	74.0	845	827	118
2	3-5	0.06	73.5	805	800	105
3	1-5	0.07	72.5	818	780	138
3	2-5	0.08	72.5	765	780	85
3	3-5	0.06	72.5	785	780	105
Average			72.3	794	771	123

## ANALYSIS OF VARIANCE FOR LOAF VOLUME RESPONSE

	Degrees of freedom	Mean squares
Granulations	4	5850.6**
Flours	2	5789.4**
Brans	2	709.8
Granulations × flours	8	552.2
Granulations × brans	8	211.8
Flours × brans	4	450.7
Flours × brans × granulations	16	334.7
Total	44	

\*\* Highly significant.

Water extracts were used to replace a corresponding amount of water in the dough mix. All doughs were then baked; the loaf volumes were as follows:

Extract	Control cc.	Control + extract cc.
Water	630	747
Water	615	730
Water	635	745
Ether	615	637
Alcohol	615	645

This indicates that an ameliorating substance must be present which is soluble in water and at least relatively insoluble in ether and alcohol. This is in agreement with the early work of Willard and Swanson (1911) and White (1913) who found that water extracts of bran increased loaf volume.

*Effect of Washed Bran and Cellophane.* Washed bran was prepared by tying coarse bran in cheesecloth and washing vigorously under running water for 3 hr. The resulting wet bran was dried as rapidly as

possible at room temperature in a forced-draft air oven. The dried bran was then ground in the Mikropulverizer and separations of different granulations were made on the experimental sifter. This material was then added to white flours and baked in the manner described for unwashed bran. As a control for this work, cellophane ground to corresponding granulations was used. The loaf volumes are given in Tables II and III. Representative photographs are shown in Figures 2 and 3.

TABLE II  
EFFECT OF WATER-EXTRACTED BRAN PARTICLE SIZE ON LOAF VOLUME

Flour	Average diameter of bran particles	Loaf volume		
		Washed bran	Control	Response (Diff. + 400)
	<i>mm</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>
1	2.49	468	718	150
2	2.49	608	810	198
3	2.49	440	740	100
Average		505	756	149
1	1.37	485	718	167
2	1.37	635	810	225
3	1.37	460	740	120
Average		527	756	171
1	0.36	550	718	232
2	0.36	713	810	303
3	0.36	613	740	270
Average		625	756	268
1	0.07	595	718	277
2	0.07	735	810	325
3	0.07	590	740	250
Average		640	756	284

ANALYSIS OF VARIANCE FOR LOAF VOLUME RESPONSE

Source of variation	Degrees of freedom	Mean square
Granulations	3	13,845**
Flours	2	6,447**
Interaction	6	475

\*\* Highly significant.

These baking data were treated to statistical analysis by subtracting the control volumes from the corresponding bran or cellophane loaf volumes and adding 400 to this figure. These response values were

TABLE III  
EFFECT OF CELLOPHANE PARTICLE SIZE ON LOAF VOLUME

Flour	Av. diameter of cellophane particles	Loaf volume		
		Cellophane	Control	Response (Diff. + 400)
	<i>mm</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>
1	1.02	547	750	197
2	1.02	497	820	77
3	1.02	480	750	130
Average		508	773	135
1	0.67	610	718	292
2	0.67	570	820	150
3	0.67	590	750	240
Average		590	763	227
1	0.27	640	725	315
2	0.27	652	784	268
3	0.27	695	750	345
Average		662	753	309
1	0.06	670	745	325
2	0.06	700	820	280
3	0.06	715	777	338
Average		695	781	314

## ANALYSIS OF VARIANCE FOR LOAF VOLUME RESPONSE

Source of variation	Degrees of freedom	Mean squares
Granulations	3	24,848.7**
Flours	2	12,357.3**
Interaction	6	1,098.2
Total	11	

\*\* Highly significant.

analyzed for variance, using the interaction, flours  $\times$  granulations, as error variance; the results are summarized at the foot of Tables II and III. The correlation coefficient between the figures for washed bran and the average diameter of bran particles was  $-0.954$ . Statistical comparison of corresponding cellophane and washed bran loaf volume figures show no significant difference between the two series.

### Discussion

Examination of the data on the chemical analyses of the brans used fails to show any factor which might consistently cause the different

granulations of bran to vary in their baking qualities on this basis alone. Examination of the analyses of variance and of the correlation coefficients between bran size and loaf volume shows that the granulation of bran plays an important role in the baking of bread containing bran. Loaf volume of these breads is negatively correlated with bran size, indicating that the size of bran loaves increases as the bran particles are ground finer. This leads to the conclusion, at present realized to some extent in commercial circles, that better whole wheat flours may

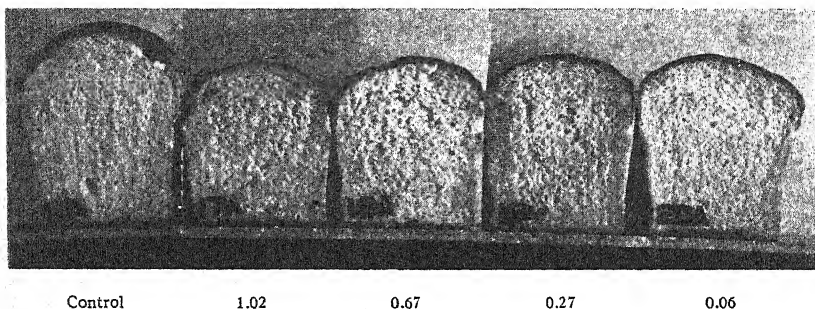


Fig. 2. Loaves of bread baked with various granulations of cellophane. Number under each loaf indicates average diameter in mm.

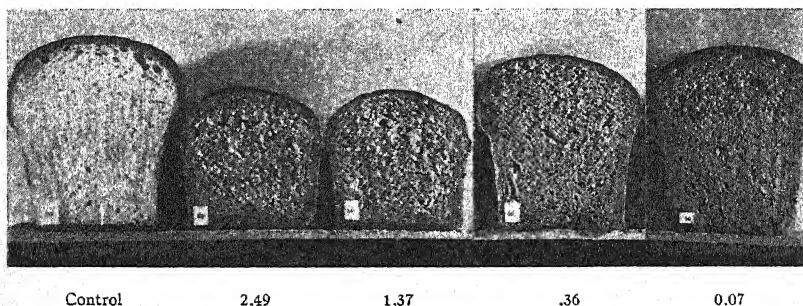


Fig. 3. Loaves of bread baked with various granulations of washed bran. Number under each loaf indicates average diameter of particle in mm.

be produced by reducing the bran contained therein to as fine a particle size as possible. In view of the possibility of starch granule injury, this may require a grinding of the bran after separation from the endosperm. A striking feature, aside from loaf volume, was the change of color and texture as the bran size became smaller. At the last level of granulation in which bran particles approached flour particle size, it became difficult to distinguish individual bran particles in the finished product, and the grain and texture were similar to those of white bread. This is shown to some extent in Figure 1.

The significant differences between flours indicate, in this series at least, a difference in response of flours to the addition of bran. On the other hand, response to different brans did not differ significantly.

An unusual feature of unwashed bran loaves is their consistently large volume as compared with the washed bran and cellophane loaves. Comparison of the washed bran and cellophane loaves shows that they do not differ significantly, indicating that washed bran has only a "diluting" effect on baking similar to an inert substance such as cellophane. However, even in these two series, the effect of granulation is clearly shown. The presence of two factors in the problem of bran-containing doughs is then indicated. One is detrimental and merely mechanical and is diminished by grinding bran to a finer particle size. The other is an improving factor which is water-soluble, as indicated both by data on washed bran loaves and by work on water extracts of bran. The authors are at present further investigating this factor.

### Summary

Three samples of bran were ground so that five different granulations were prepared from each. Granulations varied in average particle size from 0.06 mm in diameter in the finest grinding to 2.66 mm in the coarsest. These brans were superimposed at the rate of 15% on three different bread flours and the resulting mixture baked, using a slightly modified A.A.C.C. procedure.

The effect of the addition of bran on loaf volume varied with the different flours, but the three brans produced similar results.

A high negative correlation was found between loaf volume and bran-particle size. At the level used, coarse bran caused a very significant decrease in loaf volume, while the finest granulations caused a barely significant increase in loaf volume when compared to the corresponding control loaves. Bran contains a water-soluble substance which when added to white flour doughs increases loaf volume. Water-extracted bran decreases loaf volume to about the same extent as cellophane when added to flour at the same stage of granulation. Results indicate that bread of good volume and texture can be produced from flour containing finely ground bran.

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## A COMPARATIVE STUDY OF THE DEVELOPMENT OF AMYLASES IN GERMINATING CEREALS<sup>1</sup>

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Most investigations dealing with the development of amylases in germinating cereals have been limited to those cereals adapted to cool climates. For example, the nature of the amylase systems of barley and wheat malts is well understood. In contrast, information relating to the "warm climate" cereals, such as maize and sorghum, is quite limited. The present communication deals with the results found when seven cereals were germinated under comparable conditions. Four of these can be designated as cool-climate cereals—barley, wheat, rye, and oats. Three are representatives of the warm-climate group—maize, sorghum, and rice.

It is well known that the cereals barley and wheat are characterized by high levels of  $\beta$ -amylase when ungerminated and by relatively high levels of both  $\alpha$ - and  $\beta$ -amylase when germinated. The researches of Baker and Hulton (1921), Naylor, Spencer, and House (1925), Ohlsson and Uddenberg (1933), and Chrzaszcz and Janicki (1933) demonstrated that rye has amylolytic properties very similar to those of barley and wheat. In contrast, oats, either germinated or not, was found to be deficient in  $\beta$ -amylase (Baker and Hulton, 1929; Ohlsson and Edfeldt, 1933; Chrzaszcz and Janicki, 1933). However, like barley, wheat and rye, this cereal does have  $\alpha$ -amylase activity when germinated.

<sup>1</sup> Published with the approval of the Director as paper No. 344, Journal Series, Nebraska Agricultural Experiment Station.

With regard to the warm-climate cereals, Patwardhan (1929) found that germinated maize had both starch saccharifying and starch liquefying properties, but that the ratio of saccharifying activity to liquefying activity was much lower than that characteristic of barley malt. Gore and Józsa (1932), and Chrzaszcz and Janicki (1933) found that ungerminated maize had low starch liquefying properties and negligible saccharifying activity. In contrast, Bernstein (1943) advanced the hypothesis that ungerminated maize has  $\beta$ -amylase activity in small degree and a complete absence of  $\alpha$ -amylase. In a further report (Bernstein, 1943a), it was shown that germinated maize had both  $\alpha$ - and  $\beta$ -amylase activity to an appreciable extent. Sorghum exhibits amylolytic properties similar to those of maize—low starch liquefying and saccharifying properties in the ungerminated state (Patwardhan and Norris, 1928; Gore and Józsa, 1932) and a low ratio of saccharifying to liquefying or to dextrinizing activity when malted (Norris and Viswanath, 1923; Patwardhan and Norris, 1928; Acharya, 1934). Recent studies by Kneen (1944) indicate that the amylase of sorghum is primarily of the  $\alpha$  type. The  $\alpha$ -amylase was present in ungerminated sorghum in low concentrations and in much greater quantity in the germinated grain.

Many studies have been made of the amylase system of rice, and a few of these were available to the writer. Gore and Józsa (1932) found that ungerminated rice had measurable starch liquefying activity but no saccharifying activity. Karmarkar and Patwardhan (1931) and Venkata Giri and Sreenivasan (1937, 1938) likewise found that ungerminated rice was very low in amylolytic activity. In addition, they showed that a pronounced increase in starch saccharifying and liquefying properties occurred when rice was germinated. This was confirmed by Yamagishi (1938) and by Sreenivasan (1939). The conclusion drawn from the data was that germinated rice had more than one amylase; in fact, Yamagishi (1938) maintained that three independent amylases were present—a starch liquefying, a dextrinizing, and a saccharifying enzyme.

The available data show then that of the seven cereals cited, ungerminated barley, wheat, rye, and oats all have considerable starch saccharifying activity, oats being much lower in this respect than the other three. By comparison, starch saccharifying activity by extracts of ungerminated maize, sorghum, or rice is negligible. All the cereals appear to have some starch liquefying activity (Gore and Józsa, 1932), but of very low degree when compared to that characteristic of their malts. In all cases, germination of these cereals caused a marked increase in starch saccharifying and liquefying (or dextrinizing) activities. Malts of barley, wheat, and rye were found to have all these activities

in high degree, whereas the malts of oats, maize, sorghum, and possibly of rice showed low ratios of saccharifying to liquefying activities.

The general conclusion has been that all germinated cereals have both  $\alpha$ - and  $\beta$ -amylase present, but in varying degree and in varying ratio to each other. The present investigation was designed to provide additional data on the nature of the amylase systems of a number of cereals.

### Materials and Methods

Grains used for the study were wheat (Cheyenne selection), barley (Wis. ped. 38), maize (Svec Reid), sorghum (Early Kalo), rice (Blue Rose), and unidentified samples of rye and oats. Excepting the rice sample, all were grown under the same environment.

The methods used for germination, drying, sampling, and analysis were essentially those reported by Kneen, Miller, and Sandstedt (1942)—sound grain was selected, steeped to a high moisture level, germinated by the "rag doll" technique, and dried at room temperature with the aid of high speed fans. "Free" and "total" (papain) extractions were made on both the germinated and ungerminated samples. To assist in stabilizing  $\alpha$ -amylase (Kneen, Sandstedt, and Hollenbeck, 1943), dilute calcium acetate solution (1 mg/ml) was used instead of water for extractions and for dilutions. Extraction was carried out at 30°C, and when papain was used it was at a 10% level. Extraction times for the various tests are indicated.

The methods employed for evaluating the  $\alpha$ - and  $\beta$ -amylase activities of the samples were based on those proposed for barley and wheat by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941). Estimation of the  $\alpha$ -amylase activities of ungerminated cereals was made according to the procedure outlined by Kneen, Sandstedt, and Hollenbeck (1943). Calculation of the  $\beta$ -amylase activities of the malts is based on an assumption that the saccharifying potentials of the  $\alpha$ -amylases of all the cereals examined are similar to those established for wheat and barley. This has been verified for sorghum (Kneen, 1944).

### Results

*Amylases of the Ungerminated Grains.* Table I shows a comparison of the ungerminated grains. The "free" extracts were 1-hr calcium acetate extracts; the "total" were 18-hr extracts with 10% papain. In conformity with the literature it was found that the saccharifying activities of barley, wheat, and rye were high and that of oats, low. Starch saccharification by the samples of maize, sorghum, and rice was in amounts below the sensitivity of the method. Extracts of all the

TABLE I  
THE AMYLOLYTIC ACTIVITY OF UNGERMINATED CEREAL GRAINS

Cereal	Saccharifying activity		$\beta$ -amylase activity		$\alpha$ -amylase activity	
	"Free"	Total	"Free"	Total	"Free"	Total
	units	units	units	units	units	units
Barley	10.7	29.8	10.7	29.8	0.045	0.058
Wheat	7.5	25.1	7.5	25.1	0.050	0.063
Rye	9.1	17.8	9.1	17.8	0.089	0.111
Oats	0.7	2.4	0.7	2.4	0.262	0.297
Maize	*	trace	*	*	0.101	0.249
Sorghum	*	trace	*	*	0.031	0.127
Rice	*	trace	*	*	0.075	—

\* Below the sensitivity of the method used.

samples had  $\alpha$ -amylase activity in measurable quantities. The amounts of  $\alpha$ -amylase were, however, too low to influence saccharification appreciably, so that for these ungerminated samples "saccharifying units" were synonymous with " $\beta$ -amylase units."

Approximately one third of the "total"  $\beta$ -amylase of the barley, wheat, and oat samples was readily extractable with dilute calcium acetate solution and about one half of the rye  $\beta$ -amylase.<sup>2</sup> The  $\alpha$ -amylase of ungerminated wheat, barley, rye, and oats was somewhat more "soluble" than the  $\beta$  component; *i.e.*, a greater percentage of the papain-extractable enzyme was extracted in 1 hr by the dilute calcium acetate solution. The  $\alpha$ -amylase of sorghum and maize was relatively more difficult to extract. No value is given for the total  $\alpha$ -amylase of rice. Considerable difficulty was experienced with the papain extracts of this cereal and poor end-points prevented an accurate evaluation of the  $\alpha$ -amylase activity.

*Amylases of the Germinated Grains.* The germination study was conducted using 45-g samples of the grain, a 24-hr steep at room temperature, and germination at 16.5°C for 5 days. In an attempt to obtain a wider spread between the "free" and "total" amylases of these germinated samples, a 15-min calcium acetate extraction was compared with an 18-hr calcium acetate-papain extraction. The data are presented in Table II.

It is apparent from Table II that in all cases germination was coincident with an increase in  $\alpha$ -amylase content. In a comparison of the various cereals, however, it is obvious that amylase production is not proportional to sprout length. For example, the germinated oat sample had the longest sprouts but ranked fifth in  $\alpha$ -amylase production,

<sup>2</sup> It should be noted that calcium acetate solution, even one as dilute as that used, is a considerably more efficient extractant than distilled water.

TABLE II  
THE AMYLOLYTIC ACTIVITY OF GERMINATED CEREALS

Cereal	Sprout length	Saccharifying activity		$\beta$ -amylase activity		$\alpha$ -amylase activity	
		"Free"	Total	"Free"	Total	"Free"	Total
	mm	units	units	units	units	units	units
Barley	25-30	31.0	39.1	26.5	34.4	90.5	94.0
Wheat	20-30	26.4	34.4	16.5	23.7	197.3	214.7
Rye	20-30	20.1	23.6	15.4	17.6	93.2	119.8
Oats	25-35	2.2	3.0	*	*	53.1	60.3
Maize	15-20	0.24	1.2	*	*	31.1	35.6
Sorghum	10-20	1.8	1.9	*	*	73.4	75.5
Rice	2-5	0.05	0.3	*	0.2	1.4	2.3

\* Below the sensitivity of the method used.

showing less than one third the activity of wheat. As would be anticipated, the physiological activities associated with active growth brought about a release of the "bound"  $\beta$ -amylase so that the major portion of the  $\beta$ -amylase in the germinated samples was extracted without the use of papain.

The cereals, maize and sorghum, that showed no measurable  $\beta$ -amylase activity in the ungerminated state likewise showed none when germinated.<sup>3</sup> The  $\beta$ -amylase activity of the germinated rice sample was sufficient to permit evaluation but was only about one hundredth as much as that of wheat, barley, or rye.

A comparison of the data in Tables I and II demonstrates that while there was an increase in the amounts of free  $\beta$ -amylase during the germination of wheat, barley, and rye, the total  $\beta$ -amylase activities of these cereals remained relatively constant. Apparently the oat  $\beta$ -amylase largely disappeared during germination. Since germination resulted in a decrease of total dry weight with all the cereals, the  $\alpha$ - and  $\beta$ -amylase data were recalculated on the basis of the original dry weights of the ungerminated samples. This should give an estimate of "absolute" changes in enzyme contents during germination.

*"Absolute" Changes in Amylase Content.* The recalculated data for the total  $\alpha$ - and  $\beta$ -amylase contents of the germinated samples are given in Table III, together with the activities of the ungerminated samples and the dry weight losses resulting from germination. The activity of  $\alpha$ -amylase is recorded in terms of  $\alpha$ -saccharifying units (saccharifying units minus  $\beta$ -saccharifying units). When this method of calculation is used, it is seen that there was an actual loss of  $\beta$ -amylase during the

<sup>3</sup> Following the completion of the work herein reported, further investigations demonstrated that the germinated samples of oats, maize, and sorghum do have  $\beta$ -amylase activity but of a degree below the sensitivity of the method. An approximate evaluation of the  $\beta$ -amylase contents of three germinated sorghums (Kneen, 1944) showed values of 0.028, 0.093, and 0.24 units of activity. The lowest of these three values is that found for the  $\beta$ -amylase activity of the germinated Early Kalo sample used in the comparative study.

TABLE III

"ABSOLUTE" CHANGES IN THE CONTENTS OF TOTAL  $\beta$ - AND  $\alpha$ -AMYLASE DURING GERMINATION OF THE CEREALS

Cereal	Loss of dry weight by germination	Total $\beta$ -amylase		Total $\alpha$ -amylase	
		Ungerminated	Germinated	Ungerminated	Germinated
	%	units	units	sacc. units	sacc. units
Barley	21.7	29.8	26.9	trace	3.68
Wheat	23.5	25.1	18.1	trace	8.20
Rye	21.7	17.8	13.8	trace	4.69
Oats	26.4	2.4	*	trace	2.22
Maize	18.5	*	*	trace	1.45
Sorghum	19.8	*	*	trace	3.03
Rice	13.8	*	0.2	trace	0.99

\* Below the sensitivity of the method used.

germination of those grains originally showing  $\beta$ -amylase activity, this loss being approximately balanced by a corresponding gain in  $\alpha$ -saccharifying activity. As with previously recorded data for wheat (Kneen, Miller, and Sandstedt, 1942), there would appear to be some relationship between the two changes. On the other hand, those grains having no measurable  $\beta$ -amylase in the ungerminated state showed normal production of  $\alpha$ -amylase during germination and seedling growth.

*Comparative Temperature Influence on Germination.* The temperature used (16.5°C) is known to be favorable for germination of the cool-climate cereals but probably is far below the optimum for the warm-climate group. Accordingly, the seedling growth and amylase development of wheat and sorghum were compared at four temperatures, 14°, 18°, 24°, and 30°C. Germination of each pair of samples was terminated when the average sprout length of either grain attained approximately 40 mm. Table IV shows the comparative growth rates for the two cereals.

The data of Table IV show clearly the inhibitory influence of low temperature (14°C) on the germination and seedling growth of sorghum. On the other hand, germination was uniformly good at 30°C and the growth rate at this temperature greatly exceeded that of wheat. Growth rates at the intermediate temperatures of 18° and 24°C were approximately equal for the two cereals.

The amylase activities of the various samples were determined and are recorded in Table V. Data were obtained for both free (1-hr) and total (18-hr with 10% papain) extractions. In addition, the activities of the original ungerminated samples are given for comparison.

The amylase activities of those wheat samples germinated at 14°, 18°, and 24°C were very similar. This would be anticipated from the

TABLE IV  
RELATIVE GROWTH OF GERMINATING WHEAT AND SORGHUM  
AT FOUR TEMPERATURES

Days germi- nated	Approximate average length of sprout							
	14°C		18°C		24°C		30°C	
	Wheat	Sorghum	Wheat	Sorghum	Wheat	Sorghum	Wheat	Sorghum
	<i>mm</i>	<i>mm</i>	<i>mm</i>	<i>mm</i>	<i>mm</i>	<i>mm</i>	<i>mm</i>	<i>mm</i>
2	1	trace	2.5	2	4	4.5	7.5	37.5
3	2	1	9	8	12	15		
4	3.5	2	25	17.5	36	40		
5	5	2	40	40				
6	10	2						
9	45	5						

TABLE V  
RELATIVE AMYLASE ACTIVITIES OF WHEAT AND SORGHUM GERMINATED  
AT FOUR TEMPERATURES

Germina- tion time	Germina- tion tem- perature	Wheat				Sorghum		
		$\alpha$ -amylase		$\beta$ -amylase		$\alpha$ -amylase		$\beta$ -amylase
		"Free"	Total	"Free"	Total	"Free"	Total	"Free" or total
days	°C	units	units	units	units	units	units	units
9	14	250	277	18.2	18.5	31.4	31.0	*
5	18	278	293	18.7	20.4	129	126	*
4	24	250	262	19.4	19.5	109	109	*
2	30	55.3	69.8	14.5	25.9	111	113	*
Ungerminated		0.050	0.063	7.5	25.1	0.031	0.127	*

\* Below the sensitivity of the method used.

uniformity of their seedling development. Amylase production as well as growth was rapid at 24°C, but at 30°C the wheat germinated with extreme irregularity and the inhibitory effects of high temperature became evident. It is notable that there was a marked loss of total wheat  $\beta$ -amylase at the three lower germination temperatures; no loss of this component was evident during the restricted growth at 30°C.

The production of  $\alpha$ -amylase during the growth of the sorghum samples at the three higher temperatures of 18°, 24°, and 30°C was quite comparable; this, again, is in accord with the relative uniformity of sprout development. Only 2 days at 30°C were required for this development as compared with 5 days at 18°C. The inhibitory influence of the 14°C temperature on sorghum growth was further evidenced by the deficiency in  $\alpha$ -amylase development. As in the pre-

vious study, the presence of  $\beta$ -amylase in the samples of germinated sorghum could not be detected by the methods used.

It is apparent that, with one grain, conditions which influence germination and growth have a comparable effect on  $\alpha$ -amylase production. However, it is notable that a sprout elongation to approximately 40 mm was accompanied by about twice the amylase production in wheat as in sorghum. Additional data obtained with rice further confirm the observation that with different cereals equal growth rate is not necessarily coincident with equal  $\alpha$ -amylase production. A sample of rice was germinated at 30°C for 5 days. The average sprout length at the end of this period approximated 30 mm. After drying, a 1-hr extraction was made and amylase activities were determined. These were found to be 8.9 units of  $\alpha$ -amylase and 0.54 units of  $\beta$ -amylase. When compared with any of the other cereals tested (Table II), it is apparent that the growth of germinating rice coincided with relatively minor production of  $\alpha$ -amylase. These data likewise provide additional evidence that germinated rice does contain  $\beta$ -amylase, but in relatively small quantity.

### Discussion

In a general way the results presented conform to those previously reported in the literature. The chief exception is that the customary methods for the evaluation of  $\beta$ -amylase activity were not sufficiently sensitive to detect the presence of this enzyme in either maize or sorghum. However, a micromethod (Kneen, 1944) did demonstrate the presence of  $\beta$ -amylase. It appears that just as  $\alpha$ -amylase is present in cereals, germinated or ungerminated, in amounts varying from traces to considerable quantities, so the presence of  $\beta$ -amylase may be characteristic of all cereals. In some instances the levels of this latter component are so low that they cannot be evaluated by macromethods. The data presented for single samples are not entirely conclusive in themselves but become significant when supplemented with other investigations (Kneen, 1944 and unpublished data).

Other limitations of the comparative germination study warrant emphasis. Wheat appears to be particularly efficient in the production of  $\alpha$ -amylase. However, differences in growth temperature optima for the various cereals and the use of single samples do not justify such a conclusion. As a further example while oats are characteristically low in  $\beta$ -amylase, the sample used may have been abnormally low. The results then illustrate the variation which may be expected when the usual methods of amylase evaluation are applied to a miscellaneous group of cereals and to some extent emphasize the limitations of such methods.



Industrially, the findings may have considerable significance if it be assumed that the amylase systems of these germinated cereals can be duplicated by malting procedures. A malt of high potency with regard to both  $\alpha$ - and  $\beta$ -amylase could be prepared from either wheat, barley, or rye. Cereals such as oats, maize, sorghum, or rice would be of little value for the production of malts with high saccharifying activity (Lintner value) but certain ones would be adequate for the production of  $\alpha$ -amylase. If malts having only the  $\alpha$  component were desired, it appears that this could be approximated by malting a selection of sorghum low in  $\beta$ -amylase. Beta-amylase relatively free of the  $\alpha$  form can, of course, be obtained from selected samples of the ungerminated cereals. Thus cereal preparations having almost any desired ratio of the two malt amylase components can be made available for use.

### Summary

Seven cereals—barley, wheat, rye, oats, maize, sorghum, and rice—were selected, and amylase activities of both the ungerminated and germinated grains determined. Beta-amylase was found in relatively high amounts in ungerminated barley, wheat, and rye and in much less quantity in oats. The method used was not sufficiently sensitive to detect the presence of this enzyme in ungerminated maize, sorghum, or rice. From one third to one half of the total (papain-extractable) amylase was readily extracted by dilute calcium acetate solution. All the ungerminated samples had  $\alpha$ -amylase activity but in relatively minor degree.

Germination of the cereals in all cases led to a pronounced increase in  $\alpha$ -amylase activity. The  $\beta$ -amylase of barley, wheat, and rye became more readily extractable. The  $\beta$ -amylase activity of germinated rice, though low, could be evaluated, but the levels of this enzyme in germinated oats, maize, and sorghum were below the sensitivity of the customary methods.

The small quantity of  $\beta$ -amylase present in oats largely disappeared during the germination process. When "absolute" changes in amylase activities were calculated for all the samples, it became apparent that with barley, wheat, and rye, a marked loss of  $\beta$ -amylase occurred during germination.

A study of the influence of temperature on the development of amylase activity during the germination of wheat and sorghum was conducted. Temperatures normally considered desirable for malting wheat inhibited sorghum germination. The significance of this relationship in a comparison of amylase development during the germination of different cereals was pointed out.

Industrially, the availability of cereal preparations having almost any desired combination of  $\alpha$ - and  $\beta$ -amylase may have some significance. For example, a malt having high  $\alpha$ -amylase activity and practical freedom from  $\beta$ -amylase could be prepared from sorghum, and one having both  $\alpha$  and  $\beta$ -amylase from barley; the ungerminated barley itself provides a source of  $\beta$ -amylase relatively free from the  $\alpha$  form.

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## CORRELATION BETWEEN CHEMICAL STRUCTURE OF SOME ORGANIC COMPOUNDS AND THEIR ACTION AS FLOUR IMPROVERS AND AS INHIBITORS OF PROTEOLYTIC ENZYMES<sup>1</sup>

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Jørgensen (1935) has shown that those oxidizing agents which are flour improvers, such as bromates, iodates, and perborates, are also inhibitors of proteolytic enzymes of the papain type, which are now known to be present in sound wheat flour, and he also found that other oxidizing agents, such as chlorates, which are not flour improvers, do not inhibit these proteolytic enzymes. Correlation between flour improvement and inhibition of proteolytic enzymes of the papain type appears to be an established fact. It does not necessarily follow that the action of flour improvers of this type is primarily due to their action as protease inhibitors, although there is much evidence in favor of such a theory (Elion, 1943).

Some authors are more inclined to explain the action of oxidizing and reducing agents on dough by a direct effect on the flour proteins. Elion (1943) described experiments with glutathione, which showed that glutathione had a softening effect on dough made from unheated flour, but that it had no such effect on dough made from heated flour (in which the proteolytic enzymes were inactivated by the heat treatment), while papain had still a softening effect on dough made from this heated flour. These experiments cannot very well be explained if it is assumed that the action of oxidizing and reducing agents is primarily due to a direct effect on the flour proteins.

<sup>1</sup> The terms, "inhibitors of proteolytic enzymes" and "inhibition of proteolytic enzymes," have been used in this paper to avoid a longer, although more correct, description. It should be kept in mind, however, that proteolytic enzymes can be measured only by the effect which they produce. Inhibitors of proteolytic enzymes have the effect of reducing or eliminating proteolytic activity. These inhibitors may act either directly on the enzymes, or they may act indirectly by decomposing any activators of proteolytic enzymes that are present.

While prior to Jørgensen's papers on this subject only certain *oxidizing* agents were known to be flour improvers, Jørgensen found that ascorbic acid, which is a *reducing* agent, also acts both as a flour improver and as an inhibitor of proteolytic enzymes, and he stated that further investigations were required to determine whether ascorbic acid itself possesses these interesting properties, or whether it might actually be the oxidized form of ascorbic acid which is the active compound. Melville and Shattock (1938) found that dehydroascorbic acid is a more efficient flour improver than ascorbic acid and they demonstrated that wheat flour contains an enzyme capable of catalyzing the oxidation of ascorbic acid to dehydroascorbic acid.

The purpose of the present investigation has been to study whether any other organic compounds could be found which also act both as flour improvers and inhibitors of proteolytic enzymes of the papain type, and whether, in the event of positive results, any correlation between the chemical structure of such compounds and their particular action could be recognized.

In his first paper on the inhibition of proteolytic activity, Jørgensen (1935) described only the effectiveness of ascorbic acid, but in his related British patent (1936) he disclosed the action of ascorbic acid and its alkali salts as flour improvers. Noury and Van der Lande (1939), referring apparently to Jørgensen's discovery, gave a somewhat different description of the effective compounds, namely l-ascorbic acid and "its related compounds" or compounds containing "the l-ascorbic acid radical," and in the corresponding French patent (1938) they described the compounds as ascorbic acid and "other enediols." Although Jørgensen himself did not describe compounds other than ascorbic acid and its alkali salts, and Noury and Van der Lande did not present experimental evidence in support of the description "other enediols," the latter authors thus suggested a relation of enediol groups in the molecule to the action of flour improvers. In other fields than that of proteolytic enzymes, relationship of chemical structure to biological action has frequently been described in the literature.

Preliminary experiments led us to presume, contrary to the suggestion of Noury and Van der Lande, that the presence of an enediol group  $\cdot\text{C}(\text{OH})\text{:C}(\text{OH})\cdot$  does not alone determine whether a compound will act as a flour improver and further investigations have supported this viewpoint.

### Experimental

In our initial experiments the influence of certain compounds on the effect of papain was studied. A simple method to investigate whether a compound inhibits papain has been described by Jørgensen

(1935). In this method gelatin is used as the standard protein material and a papain extract is allowed to react for some time upon a properly buffered gelatin solution at a constant temperature, in the presence or absence of the compound which is to be investigated. Thereafter it is observed whether the gelatin still possesses its property to solidify in a refrigerator.

The papain extract was made by extracting 1 g of papain (Witte) with 100 ml water for 30 min at a temperature of 35°C, whereupon the extract was filtered and the filtrate used. The reaction mixture was made up as follows: 10 ml gelatin solution (made from 20 g gelatin powder to 250 ml water) + 6 ml Sørensen's citrate buffer (pH = 4.95) + 2 ml water (or water and solution of the compound under investigation) + 2 ml papain filtrate. The mixture was kept 1 hour at a temperature of 40°C and then placed in a refrigerator and observed after 15, 30, 45, and 60 min and also the next morning.

The first compound used in these experiments was reductone, which is a strong reducing compound. It proved to be very effective in inhibiting the proteolytic action of papain on gelatin. It was found necessary to use freshly made solutions of reductone, since at the very low concentrations involved, appreciable decomposition occurred in 24 hours.

As stated previously, some doubt arose as to whether the presence of an enediol group in the molecule would be decisive in determining whether a compound will inhibit papain; rather it was presumed that the combination of an enediol group and a carbonyl group next to it:  $\cdot\text{C}(\text{OH})\text{:C}(\text{OH})\cdot\text{CO}$ . would be the determining factor. As a matter of fact, this latter group is present both in ascorbic acid and in reductone and an endeavor was made to investigate such compounds that possess an enediol group, but do not have a carbonyl group next to this enediol group. In the event that our presumption were correct, compounds containing an enediol group only should not inhibit papain, whereas compounds having an enediol group next to a carbonyl group should inhibit papain.

Some difficulty was encountered in obtaining compounds having an enediol group only, particularly among the aliphatic compounds. Dimethylacetyleneglycol is such a compound. It is the desmotropic form of acetoin and is believed to be present in small quantity in, and in equilibrium with, acetoin. Experiments with acetoin were entirely negative; the action of papain on gelatin was not inhibited.

The following cyclic compounds were investigated: pyrocatechol, pyrogallol, gallic acid, adrenalin, haematoxylin, carminic acid, and purpurin. While some of these were only partially soluble in water, none of them inhibited papain. All these compounds have one or

more enediol groups in the formula, but no carbonyl group next to the enediol group.

Compounds which have an enediol group adjacent to a carbonyl group were then investigated. Besides reductone, which has already been stated to be a strong inhibitor of papain, the following compounds were tested: dihydroxymaleic acid, reductic acid, and tetrahydroxyquinone. All of these strongly inhibited the proteolytic action of papain, and it was surprising to find that it was immaterial whether the compound was aliphatic or cyclic.

The gelatin method makes a rough quantitative comparison of the effectiveness of different compounds possible, since it permits the use of varying quantities of an inhibitor to determine the minimum amount of each required to inhibit the papain entirely. Several compounds were tested in different concentrations. Under the experimental conditions prevailing, the minimum molar concentrations roughly required to inhibit the papain, so that gelation occurred after one hour in the refrigerator, are given in Table I.

TABLE I

MINIMUM MOLAR CONCENTRATIONS OF DIFFERENT INHIBITORS REQUIRED ENTIRELY TO INHIBIT PAPAIN

Inhibitor	Molar concentration
Potassium bromate	0.0015
Ascorbic acid	0.00015
Reductone	0.00015
Dihydroxymaleic acid	0.0015
Reductic acid	<sup>1</sup>
Tetrahydroxyquinone	<sup>1</sup>

<sup>1</sup> Limits not determined, but less than 0.0015.

In view of these experimental results, it would not be surprising if all compounds containing the group  $\cdot\text{C}(\text{OH})\text{:C}(\text{OH})\cdot\text{CO}_2$ , whether aliphatic or cyclic, would inhibit proteolytic enzymes of the papain type. Some examples of such compounds which were not available at the time of our experiments are oxytetroneic acid and rhodizonic acid. Owing to the instability of many of the enediol compounds, no definite series has been used.

It appears to be essential that the group  $\cdot\text{C}(\text{OH})\text{:C}(\text{OH})\cdot\text{CO}_2$  be present as such. Substitution, such as obtained by replacing a hydrogen atom of the hydroxyl group by sodium, appears to eliminate the compound's property to inhibit papain. Thus, disodiumtetrahydroxyquinone has been found to be inactive, while tetrahydroxyquinone was very active.

In agreement with the proteolytic enzyme theory of Jørgensen

(1935) and of Balls and Hale (1936), compounds having the enediol group next to a carbonyl group were found not only to inhibit papain, but also to act as chemical flour improvers of the bromate and ascorbic acid type. It needs no further emphasis that not all such compounds are permissible in bread, since they may have possible poisonous effects, or have an undesirable influence on other bread properties, such as crumb color (tetrahydroxyquinone).

As an example, baking tests made with reductone will be cited. The flour used was an untreated high protein straight grade Texas flour with 15.0% protein and 0.45% ash. The dough ingredients, based on the flour weight, were yeast 2.2%, salt 1.5%, sugar 3%, shortening 1.5%, water 59%. The fermentation time was 145 min at 30°C (first punch after 75 min, second punch after 45 min and molding after an additional 25 min). The doughs were proofed 55 min at 30° and baked for 35 min at 220°. The addition of 0.0002% of reductone based on the flour weight caused an increase in the bread volume of more than 7%; moreover the bread made with reductone had a better texture, lighter crumb color, and better crust color than the control bread made without reductone.

Further investigations will be required to explain why the presence of the group  $\cdot\text{C}(\text{OH})\text{:C}(\text{OH})\cdot\text{CO}$ . in the molecule gives a compound the property of inhibiting proteolytic enzymes of the papain type and acting as chemical flour improvers. Further research will also be required to determine whether the compounds are effective themselves, or whether it is actually some oxidized form which is functioning. Also, the effect of the different compounds described above should be studied with various flours under varying baking conditions. Although it is realized that the present study is not yet conclusive and should be extended in many directions, it is thought that publication at this time may stimulate further research on the mode of action of reducing agents such as glutathione and of flour improvers of the bromate and ascorbic acid type.

### Summary

Organic compounds, whether aliphatic or cyclic, which contain the group  $\cdot\text{C}(\text{OH})\text{:C}(\text{OH})\cdot\text{CO}$ ., were found to act as inhibitors of papain. Examples of such organic compounds, some with strong reducing properties, are given and they were found to act similarly to ascorbic acid. In agreement with the proteolytic enzyme theory of Jørgensen and of Balls and Hale, it has been found that such compounds also act as flour improvers, although their use as flour improvers may be impossible for health—or other reasons.

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## AN INSTRUMENT FOR DETERMINING THE COMPRESSIBILITY AND RESISTANCE TO SHEAR OF BAKED PRODUCTS

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A number of instruments have been developed to determine the compressibility of bread. Platt (1930) describes a balance type of instrument. L. H. Bailey (1930) describes a simple apparatus in which a sample of bread is compressed in a small box by a standard weight and the compression measured. Platt and Powers (1940) describe the Baker compressibility apparatus in which a stress is applied to the sample by the use of an electric motor and the resulting strain measured. Nikolayev (1941) describes an instrument in which a weight is applied to the surface of a sample and the depression of a semispherical testing finger is measured. All of these instruments, with the exception of the Baker compressibility apparatus, use a fixed weight acting for a fixed time, and to obtain the best results it may be necessary to vary the stress with different samples.

### History

An instrument which has been in use in our laboratory for a number of years for determining the compressibility and, also, the resistance to shear of baked products will be described in the present paper. Since



it is almost automatic in operation, a relatively unskilled operator can make tests rapidly and with little error.

This instrument, in its original form, was developed by Bonney, Clifford, and Lepper (1931) to test the softness and ripeness of fruits and vegetables.

### Construction and Operation

The construction of the original instrument provided for a vertical shaft, on the bottom of which could be used needles of various sizes or a small rigid plate. Weight was applied to the shaft by delivering mercury at a constant rate into a container on a platform on top of the shaft. The depression of the needle or plate was amplified by an indicator and measured on a scale graduated from 0 to 52. The contact with this indicator was shown by a buzzer and a light operated by flashlight batteries.

Haas<sup>1</sup> (1931) modified the instrument by using a larger rigid plate on the bottom of the shaft and employed it for determining the staling rate of bread. In his work, he used a fixed depression and a varying weight delivered at a constant rate. King, Morris, and Whiteman (1936) used the original instrument for testing the compressibility of cake, employing a varying weight acting for a definite time.

Further modifications have been made which increase the accuracy of the test and the ease of operation (Fig. 1). Mercury was found to be objectionable as a weight, since there was always some spray which prevented using the slices for flavor tests later. The mercury cut-off was operated by hand. Lead shot has been substituted for the mercury, and an electric cut-off added. The flow of shot is adjusted to a standard rate of 500 g per min. Instead of flashlight batteries, 110 volt current is used. The electric cut-off consists of a simple valve held open by a solenoid. A pilot bulb, in series with the solenoid, glows until the contact is made at the lowest limit of the depression. When contact is made, the solenoid current is broken, the pilot light goes out, and the flow of shot is automatically stopped. The compression disk is attached to the shaft by a ball-and-socket joint. This arrangement has been found to give more uniform results, particularly in the case of twisted bread or where molding streaks occur. The ball and socket allows the disk to tilt and tends to average out variations in the firmness of the slices. The disk is 54 mm in diameter and is the largest that could be used on all of the samples tested without covering any of the firmer area close to the crust.

*Measuring Compressibility.* In testing compressibility, the instrument can be used in three ways: a fixed weight may be allowed to act

<sup>1</sup> L. W. Haas, W. E. Long and Company, Chicago, Ill. Private communication.

for a fixed time and the depression measured; a variable weight may be allowed to act for a fixed time and the depression measured; or the weight required to produce a fixed depression may be determined. The last method has been the most satisfactory. Either hand-sliced or commercially sliced bread can be used. In the case of unsliced bread, slices 25 mm thick are cut in a jig and the crusts trimmed off. The trimmed slice is placed under the disk which is lowered upon it. The indicator is moved to the lower part of the scale and the platform

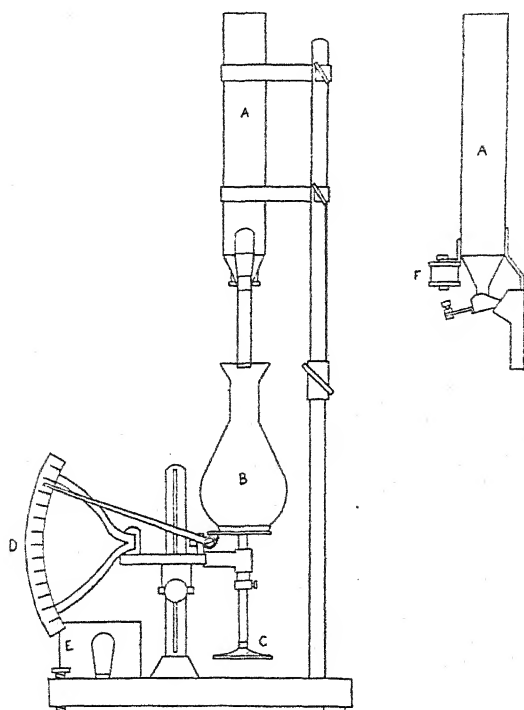


Fig. 1. Diagram of apparatus equipped with compression plate. A—Shot container; B—Receiver; C—Compression disk; D—Scale; E—Relay; F—Solenoid.

adjusted till contact is indicated by the pilot light. The indicator is then set back any desired number of divisions; 25 divisions are satisfactory for slices which are 25 mm thick. This setting corresponds to a depression of the shaft of 4.33 mm. The lever of the shot valve is then raised and the solenoid holds the feed open until contact is again made by depression of the disk. The shot is then weighed. The weight of the disk and shaft assembly is added to that of the shot and container to obtain the total weight effecting the depression. While one slice is being tested, the next may be prepared. Forty mm of each

end of the loaf are discarded. It is preferable to test more than one loaf and average the results. The instrument in use for testing compressibility is shown in Figure 2. Typical figures on the changes in

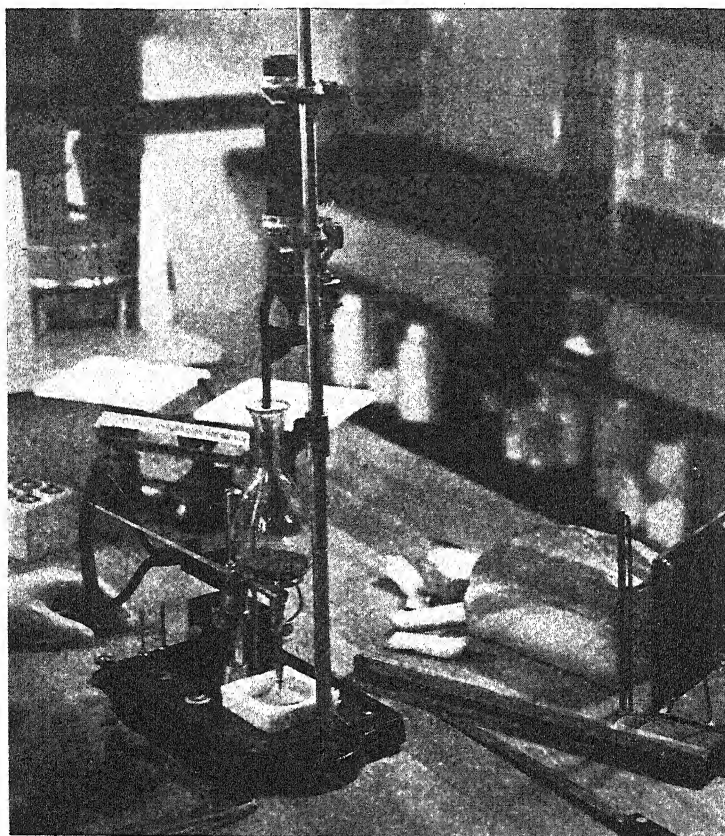


Fig. 2. Instrument in use for testing compressibility.

compressibility of bread during staling are as follows:

Formula No.	Average compressibility per slice at given intervals after baking	
	24 hr g	48 hr g
1	467	626
2	536	703
3	398	529
4	379	532

*Testing Shortness of Doughnuts.* The same principle of operation has also been used to test the shortness of doughnuts. To obtain uniform results, it has been found necessary to destroy the differences in

cell structure, since some doughnuts are quite close in texture and others are very open. For this purpose, a cylinder with a removable bottom and a telescoping cap is used. The cylinder has a diameter of 25 mm and a depth of 32 mm. The cap has a hole in the top 6.5 mm in diameter. A section of the doughnut weighing 15 g is cut in one piece and inserted in the cylinder, distorting the section as little as possible. Readings are then made through the hole in the top of the cap, using a needle 4.5 mm in diameter.

*Testing Resistance to Shear.* This apparatus has also been used with another attachment (Fig. 3) for testing the resistance to shear of

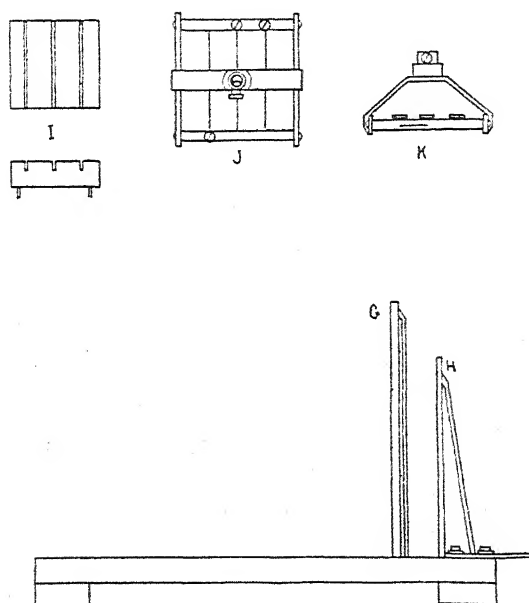


Fig. 3. Shear test equipment and jig for cutting slices. I.—Shear test base; J-K—Shear test head; G—Knife guide; H—Adjustable plate.

bread or other baked products. A frame  $3 \times 3$  inches is rigidly attached to the bottom of the shaft. Across this frame are strung three piano wires, spaced three-fourths inch apart. With the frame is used a platform five-eighths inch high. The top of the platform is one-eighth inch thick and has three slots coinciding in spacing with the wires. These slots extend through the top of the platform and down one-eighth inch into the end supports. With this arrangement, the scale indicator is not used, but only a flexible contact. A slice of bread is placed on the platform and the shot valve opened. When the wires cut through the slice, sufficient drop is obtained to make contact and shut off the feed. Either commercially sliced or hand-sliced bread

can be used. If the bread is hand-sliced, slices 15 mm thick are used. The slices are trimmed exactly 50 mm wide and approximately 60 mm long. The slice is placed on the platform with the wires running across the width. This gives a cut of 150 mm for the three wires.

### Summary

An instrument is described which can be used for testing both the compressibility and the resistance to shear of baked products. The method of operation is outlined and typical results obtained in measuring the compressibility of bread are given.

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## STUDIES ON THE FRACTIONATION OF ZEIN<sup>1</sup>

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From a study of the solubility behavior of zein with respect to ethyl and amyl alcohol, Donard and Labbé (1902, 1903) grouped zein into three types of proteins:  $\alpha$  zein—soluble in amyl and ethyl alcohol;  $\beta$  zein—soluble in ethyl alcohol only;  $\gamma$  zein—insoluble in either alcohol. From a study of the optical rotation of  $\alpha$  and  $\beta$  zeins so defined, Lindet and Ammann (1907) concluded that these represent distinct proteins. Watson, Arrhenius, and Williams (1936) obtained, by successive additions of water to a 70% ethanol solution of zein, three distinct and nearly homogeneous fractions. In a later study, Elliott and Williams

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<sup>1</sup> Paper No. 2168, Scientific Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis submitted by Robert Theis MacDonald to the faculty of the Graduate School, University of Minnesota, in August, 1941, in partial fulfillment of the requirements for the degree of doctor of philosophy.

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(1939) fractionated corn gluten by successive extractions with methanol and 70% aqueous ethanol.

Zein shows a high solubility in methyl- and ethyl-cellosolve. The present paper deals with the separation of zein into fractions by the successive additions of water to cellosolve solutions of the protein. A study was then made of some of the physicochemical properties of the fractions obtained in this manner.

Since experiment showed that the fractionation of zein by addition of water to solutions of the protein in either methyl or ethyl cellosolve yielded fractions which were entirely comparable to each other, this report will deal only with fractions obtained in the following manner from methyl cellosolve solutions.

### Experimental

*Preparation and Purification of Zein Fractions.* A sample of commercial zein <sup>4</sup> was first purified by an adaptation of the method of Mason and Palmer (1934), wherein the zein was dissolved in 80% ethanol, washed repeatedly with ethylene dichloride or petroleum ether until all color was removed and, after concentrating the solution by removal of part of the alcohol by vacuum distillation, precipitated by dilution in a large volume of 1% sodium chloride solution. The gummy mass so obtained was washed with water to remove part of the salt and the remaining alcohol and was allowed to dry in the air.

When this air-dry zein (moisture content approximately 4%) was suspended in methyl cellosolve, a part of the protein (about 10%) failed to go into solution. This fraction (called Fraction X) was removed by centrifuging and the remaining supernatant solution diluted with solvent to form an approximately 6% (by weight) solution of the protein in methyl cellosolve.

Aliquots of this solution were diluted with various amounts of water and the quantity of protein precipitated was determined. Table I

TABLE I  
EXTENT OF PRECIPITATION OF PROTEINS FROM 6% ZEIN SOLUTION IN METHYL CELLOSOLVE BY ADDITION OF WATER IN VARYING AMOUNTS

Percent (by volume) water added	Percent of total protein precipitated
23.0	0
28.5	13.2
33.3	69.6
37.5	91.6
44.4	97.5
50.0	97.7

<sup>4</sup> Commercial zeins were supplied for this work by the American Maize Products Company and by the Corn Products Refining Company.

shows the extent of this precipitation as increasing percentages of water (by volume) were added to the cellosolve solution. It is seen that approximately one third of the soluble protein is precipitated at 31% water content and approximately two thirds is precipitated at 34% water content. These water contents were chosen, then, in order that the total soluble zein should be roughly fractionated into three equal parts. The fraction precipitating at 31% water content was called Fraction A, that removed by increasing the water content to 34% was Fraction B, and that remaining in solution at 34% water content but which could be precipitated by pouring this solution into a large volume of 1% sodium chloride solution was called Fraction C. A number of such fractionations were made and parts of each fraction were repurified by suspending them in water and electrodialyzing nearly free of ash. The protein was collected and air dried, then dissolved in 80% ethanol and precipitated by pouring the concentrated alcohol solution into ethyl ether. The precipitated protein was then dried by washing with anhydrous ethyl ether several times. During this purification process a considerable and varying amount of the original fraction was lost in each case.

It was assumed that during the process of repurification there was no change in such physical properties as micellar weight, optical rotation, peptization, and the like. Table II shows the yields of finally

TABLE II  
YIELDS OF REPURIFIED FRACTIONS OF ZEIN FROM ORIGINAL FRACTIONS

Fraction	Weight	
	Before purification	After purification
X	37	20
A	60	32
B	54	26
C	58	41
A <sub>1</sub>	25	11.5
A <sub>2</sub>	17	7.5
A <sub>3</sub>	30	27.5

purified samples obtained from original amounts of each fraction so treated. It was on such repurified samples of the various fractions that the subsequent studies were made.

A further fractionation was made on Fraction A obtained as above described. An air-dry sample of this fraction (not repurified) was suspended in methyl cellosolve to form a 6% solution. Approximately 25% failed to go into solution. This was separated and designated Fraction A<sub>1</sub>. The remaining solution was brought to 31% (by volume)

of water, the water content at which all of Fraction A had separated in the original fractionation. Only a part of the redissolved Fraction A precipitated at this point. This was collected as Fraction A<sub>2</sub>, and the protein left in solution at this water content was collected as Fraction A<sub>3</sub>. Each of these three fractions of Fraction A was repurified as already described for the original fractions, and used in the following studies. Amounts of repurified Fractions A<sub>1</sub>, A<sub>2</sub>, and A<sub>3</sub> obtained from the noted amounts of the original unrepurified fractions are given also in Table II.

The nitrogen and ash contents of these fractions of zein, calculated on a moisture-free basis, are given in Table III.

TABLE III  
ASH AND NITROGEN CONTENTS OF THE VARIOUS FRACTIONS OF ZEIN  
(Results expressed on moisture-free basis)

Fraction	Ash	Total nitrogen (ash-free basis)
	%	%
X	0.18	15.68
A	0.31	15.68
B	0.15	15.77
C	0.92	15.26
A <sub>1</sub>	0.90	12.98
A <sub>2</sub>	1.93	14.69
A <sub>3</sub>	0.41	15.77

*Precipitation Studies.* A comparative study was made of the precipitating action of water upon stock solutions of the various zein fractions dissolved in methyl cellosolve (the concentration of protein in these stock solutions is shown in Table IV). In each set of experiments a definite amount of the protein solution was placed in each of a series of test tubes. A definite volume of water was carefully added, equilibrium allowed to be established, and the percent protein precipitated determined by nitrogen analysis of the supernatant solutions. Results are shown in Table IV.

The results shown in section B of Table IV are especially significant. The precipitation curves of Fraction A<sub>2</sub> and A<sub>3</sub> on the one hand, and that of Fraction A<sub>1</sub> on the other hand, point to the existence of two distinct types of protein which, for convenience, will be called Type I (Fractions A<sub>2</sub> and A<sub>3</sub>) and Type II (Fraction A<sub>1</sub>). The action of added water upon methyl cellosolve solutions of both A<sub>2</sub> and A<sub>3</sub> was very sharp, forming compact coagulums; the addition of water to Fraction A<sub>1</sub> formed a suspension which was stable even upon standing for several hours. Amounts of precipitate recorded refer actually to the amounts of separable coagulum and do not include the material left in suspension as a stable sol.



TABLE IV  
FRACTIONAL PRECIPITATION OF VARIOUS ZEIN SAMPLES BY THE ADDITION  
OF WATER TO THEIR METHYL CELLOSOLVE SOLUTIONS

Fraction	Conc. zein in stock solns.	Percentage of total protein precipitated upon additions of given percentages of water (by volume)					
		28.5	33.3	37.5	41.2	44.4	50.0
	<i>g/100 ml</i>						
Section A							
X	5.4	0	28.7	67.6	82.7	84.5	73.0
A	4.7	0	38.7	78.3	88.6	—	88.8
B	5.5	0	30.0	73.2	78.8	50.9	17.2
C	5.5	0	00.0	47.9	87.0	96.0	99.0
Section B							
A <sub>1</sub>	4.8	0	1.0	3.5	3.6	2.0	2.0
A <sub>2</sub>	4.7	0	48.1	82.9	93.8	96.1	99.0
A <sub>3</sub>	5.7	0	32.6	75.9	89.6	96.0	98.9
Section C							
Mixture, 5A <sub>3</sub> + 1A <sub>1</sub>	6.0	0	7.5	64.7	81.7	82.2	27.5
B <sub>1</sub>	4.3	0	50.2	83.1	93.8	91.8	94.9
B <sub>2</sub>	3.4	0	0	8.1	4.9	3.5	10.6

If it is assumed that in a mixture of the two types of protein (I and II) in solution, a complex disperse-phase system is formed, then, upon addition of water to such a system, the type of precipitation curve obtained would depend upon the relative quantity of each type present. The following experiment was devised to test the effect of one type upon the other with regard to the character of coagulum formed when their methyl cellosolve solutions were diluted with water.

A mixture of Fractions A<sub>3</sub> and A<sub>1</sub> was dissolved in the ratio of 5 : 1 in methyl cellosolve. Water was added to this stock solution as before. Results are shown in Table IV, section C. In this case, a mixture has been produced which is quite similar to—if not identical with—Fraction B. It should be possible, therefore, to resolve Fraction B into sub-fractions which would have the precipitation properties characteristic of Type I and Type II.

To effect such a resolution, a 6% solution of Fraction B in methyl cellosolve was prepared and water added up to 40%. The precipitate, called Fraction B<sub>1</sub>, which should be of Type I, was separated from the filtrate, which then contained Fraction B<sub>2</sub>. Each fraction was air dried and resuspended in methyl cellosolve. The results of precipitation experiments on both of these fractions are given in Table IV, section C, and may be compared with the original Fraction B. It is seen that Fraction B<sub>1</sub> gives a precipitation curve which is quite similar to that of Fraction A<sub>2</sub> or A<sub>3</sub> (Type I) while Fraction B<sub>2</sub> is similar to Fraction A<sub>1</sub> (Type II).

*Optical Rotations of Solutions of Fractions of Zein in Ethanol.* Lindet and Ammann (1907) found that the  $\alpha$  and  $\beta$  zeins, as defined by Donard and Labbé (1903), showed varying specific optical rotations when dissolved in ethanol solutions of varying water content. Also, the rotations of the two fractions were not identical when dissolved in the same ethanol-water mixture. From this they concluded that these zeins represented distinct proteins. Table V gives the  $[\alpha]_D^{25}$  values for

TABLE V

SPECIFIC OPTICAL ROTATIONS OF ZEIN FRACTIONS  $A_3$ , B, C, AND  $A_1$  AT 23°C IN DIFFERENT CONCENTRATIONS OF ETHANOL

(Values for  $\alpha$  and  $\beta$  zein by Lindet and Ammann (1907) for comparison)

Ethanol concentration	Specific optical rotation $[\alpha]_D^{25}$					
	Lindet and Ammann		Zein fractions			
	$\alpha$ zein	$\beta$ zein	$A_3$	B	C	$A_1$
%	degrees	degrees	degrees	degrees	degrees	degrees
70	29.6	40.0	39.4	36.0	32.5	23.9
80	29.6	40.0	35.8	—	32.5	23.9
90	23.1	22.1	23.3	25.2	21.7	18.3

four of our fractions together with those of Lindet and Ammann for their  $\alpha$  and  $\beta$  zeins. Fractions C and  $A_3$  give values which agree fairly well with those for the  $\alpha$  and  $\beta$  fractions, respectively, of Lindet and Ammann. Fraction  $A_1$ , however, must be considered as distinctly different from their fractions. Lipatov and Putilova (1936), in their study of gelatin fractions, found the less soluble (larger molecular) fractions showed correspondingly higher specific rotations. We should expect Fraction  $A_3$  to have a higher molecular weight than Fraction  $A_1$  on this basis. Osmotic pressure measurements showed this to be the case.

*Osmotic Pressure Studies.* Osmotic pressures of Fractions  $A_3$ , B, C, and  $A_1$  dissolved in methyl cellosolve were determined at three concentrations. The instrument used was similar to that described by Dobry (1935). As collodion is soluble in methyl cellosolve, it was necessary to use a denitrated collodion membrane. Denitration was accomplished by the use of ammonium hydrogen sulfide according to the method described by Jilk (1937). Experimental data are shown in Table VI and Figure 1.

The relationships observed between osmotic pressure and concentration for the various fractions of zein conform with the expression

$$P = AC + BC^2,$$

TABLE VI  
OSMOTIC PRESSURE DATA OBTAINED WITH ZEIN FRACTIONS B, C, A<sub>1</sub>, AND A<sub>3</sub> IN  
METHYL CELLOSOLVE SOLUTION

Protein concentration g/liter soln	Osmotic pressure <sup>1</sup> cm water (25°C)	Osmotic pressure/conc.
FRACTION C		
19.15	14.10	0.78
9.58	7.73	0.81
4.79	4.04	0.84
FRACTION A <sub>3</sub>		
19.36	18.02	0.93
9.68	7.40	0.76
4.84	3.13	0.65
FRACTION A <sub>1</sub>		
9.43	6.28	0.67
4.72	4.16	0.88
2.36	2.31	0.98
FRACTION B		
19.25	18.69	0.97
9.63	7.49	0.78
4.81	3.39	0.71

<sup>1</sup> Obtained by multiplying the difference in hydrostatic levels between solution and solvent by the density of solution.

where  $P$  is the osmotic pressure,  $C$  the concentration, and  $B$  and  $A$  are constants which represent, respectively, the slope and intercepts of straight lines obtained when  $P/C$  is plotted against  $C$ . Although the micellar weights are calculated from values of the intercepts, the fractions also appear to be characteristically differentiated by the slopes so obtained. Thus Fractions A<sub>3</sub> and A<sub>1</sub> which show marked differences with respect to specific optical rotation and precipitation behavior are likewise sharply marked in their osmotic behavior by a reversal in sign of slope. The method of least squares was used to determine the most probable value of the intercept. The intercept represents the limiting value of  $(P/C)_{C \rightarrow 0}$ . The molecular weight is then given by the expression

$$M = \frac{22.41 \times (1 + .00367 t)}{9.651 \times 10^{-4} \times \text{intercept}}$$

where  $t$  is expressed in degrees centigrade,  $P$  is expressed in centimeters of water, and  $C$  is expressed in grams of solute per 1000 ml of solution. Table VII gives the intercepts found and the corresponding calculated molecular weights.

### Discussion of Results

In the precipitation study it was found that Fraction B consists of a mixture of Type I and Type II proteins. Upon calculating the

molecular weight for a mixture composed of 85% of component of molecular weight 45,000 (as for Fraction A<sub>3</sub>) and 15% of component of molecular weight 23,330 (as for Fraction A<sub>1</sub>), a value of 41,750 is obtained. This corresponds closely to the observed value of 41,650

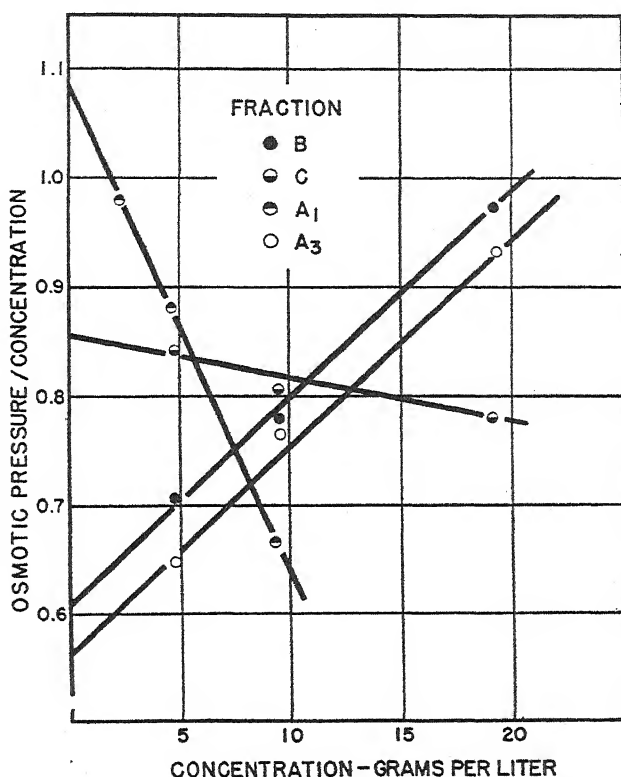


Fig. 1. Osmotic pressure-concentration relationships for various fractionated and refractionated protein products obtained from methyl cellosolve solutions of zein. Fractions B and C are originally obtained fractions, whereas Fractions A<sub>1</sub> and A<sub>3</sub> are refractionated products obtained from Fraction A.

actually found for Fraction B. It may be concluded that Fraction B consists of Type I and Type II proteins in the ratio of 85 to 15.

One of the surprising results of the osmotic pressure studies is the large difference in molecular weight between zein Fractions B and C

TABLE VII  
MOLECULAR WEIGHTS OF ZEIN FRACTIONS A<sub>3</sub>, B, C, AND A<sub>1</sub>

Fraction	Intercept	Molecular weight
A <sub>3</sub>	0.563	45,030
B	0.609	41,650
C	0.855	29,650
A <sub>1</sub>	1.087	23,330

(Fig. 1). This indicates that the separation of these two fractions has been rather sharp and distinct. The osmotic pressure studies have further corroborated the results obtained with optical rotation and peptization studies. By assuming no change in physical properties during the repurification process and that zein Fractions C, A<sub>2</sub>, and A<sub>3</sub> are free of any material similar to Fraction A<sub>1</sub> (Type II protein); also, that 15% of Fraction B, 10% of Fraction X, and 5% of Fraction A (rough approximations based upon peptization data) consist of a component such as Fraction A<sub>1</sub>, it can be estimated on the basis of yields of air-dry fractions that 65% of the total protein in zein is a fraction of molecular weight 45,000, 25% is a fraction of molecular weight 30,000, and 10% is a fraction of molecular weight 23,350.

### Summary

A method is described of fractionating zein in methyl cellosolve solution by addition of water wherein several fractions have been obtained. One of these fractions (Fraction A) was subjected to a process of refractionation which resulted in a further set of subfractions.

Precipitation studies on the various fractions indicated that two types of protein are present in zein: Type I, which is hydrophobic, is precipitated as a compact coagulum, whereas Type II, which is hydrophilic, is not precipitable by water but forms a stable suspension when thrown out of clear solution. These two types of zein protein were found to differ markedly in their total nitrogen content.

Optical rotation studies on four of the zein fractions pointed to the existence of at least three components.

Osmotic pressure studies on these same zein fractions indicated considerable variation in their molecular weights. These results, together with information obtained from peptization studies, and from the weights of individual fractions obtained in the original separation, lead to the conclusion that zein contains components which fall roughly into three groups: (1) 65% of the total protein consists of a fraction of molecular weight 45,000 (this is slightly higher than the value of 38,000 reported by Elliott and Williams, 1939); (2) 25% of the total protein is a fraction of molecular weight of 30,000; (3) 10% of the total protein is a fraction of molecular weight 23,350. The third protein fraction is of Type II whereas the others are of Type I.

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## BOOK REVIEWS

### Cellulose and Cellulose Derivatives. Volume V. High Polymers.

Emil Ott. xx and 1176 pp. Interscience Publishers, Inc., New York, N. Y. 1943. Price \$15.

This volume is a monograph written by 35 specialists located in the United States. In the introduction, the editor states: "The object of this book is to have in a unified presentation the most important modern scientific and technical information concerning cellulose and its derivatives and to have this information in such form that it becomes a thorough introduction for work on any cellulose problem by any person with reasonably wide general technical training." The historical approach has been almost entirely neglected and only sufficient technical detail has been included as was considered essential for a broad and reasonably thorough understanding of the whole field.

The contents are arranged into 10 chapters, each with many subdivisions. The chapter outline which follows shows the logical order of treatment of the various topics and indicates the scope of the volume.

*Chapter I. Occurrence of Cellulose*—significance of terms, formation of cellulose, identification of cellulose, industrial uses of cellulose, sources of cellulose, natural occurrence of combined cellulose.

*Chapter II. Chemical Nature of Cellulose and Its Derivatives*—historical survey, chain structure, end groups, chemical significance of polymolecularity, nature of the association between carbohydrate and lignin in wood, influence of hemicelluloses in pulps, base-exchange properties, cellulose tests, degradation of cellulose.

*Chapter III. Structure and Properties of Cellulose Fibers*—X-ray examination, structure of wood, microscopic investigation, mercerization, the distribution of sub-microscopic metal crystals in fibers, beating and hydration of paper fibers, sorption of water and other vapors by cellulose.

*Chapter IV. Carbohydrates Normally Associated with Cellulose in Nature*—sugars found in hemicelluloses and related polysaccharides, hemicelluloses, pectin.

*Chapter V. Lignin and Other Noncarbohydrates*—occurrence, determination, and physical properties of lignin; isolation of lignin; structure of lignin; nature of the other noncarbohydrate compounds in wood and straw.

*Chapter VI. Preparation of Cellulose from Its Natural Sources*—wood pulp, cotton linters, rags, bast fibers, straw, grasses, and similar materials.

*Chapter VII. Bleaching and Purification of Cellulose*—bleaching of cellulose textiles and rags, bleaching of acid-cooked wood pulp, multi-stage bleaching, chlorination followed by hypochlorite bleaching, bleaching of alkaline-cooked pulps, washing of pulp, miscellaneous bleaching agents and processes, bleaching and purification of pulp for special purposes, literature reviews and acknowledgment.

*Chapter VIII. Derivatives of Cellulose*—kinetics and equilibria involved in cellulose reactions, inorganic esters, cellulose organic acid esters, alkali and other metal derivatives, cellulose ethers, xanthates.

*Chapter IX. Physical Properties of Cellulose and Its Derivatives*—solubility, thermodynamic properties of solutions of long-chain compounds, determination of molecular weight, influence of polymolecularity on physical properties, theory of the viscosity of dilute solutions of long-chain compounds, practical applications of viscosity, elasticity, and strength.

*Chapter X. Technical Applications of the Physical Properties of Cellulose and Its Derivatives*—natural cellulose, regenerated cellulose and rayon, cellulose derivatives, future applications.

Many of the contributors are engaged in industry or working in research institutions supported by industrial organizations so that the presentation of industrial processes is up-to-date. Recent references which provide an adequate description of the topic under consideration have been given preference over earlier references. Author and subject indices are included.

This volume sets a high standard, and the contributors, editor, and publishers are to be congratulated in providing a modern presentation of the chemistry and technology of cellulose which will be an invaluable reference work for several years to come.

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**The Constituents of Wheat and Wheat Products.** By C. H. Bailey. 332 pp. Reinhold Publishing Corporation, New York. 1944. Price \$6.50.

In his introduction the author says that this book is intended to cover "what might be termed the descriptive biochemistry of wheat and wheat products," and that it is merely "a report of progress." The reader will soon discover that the book is, essentially, a complete historical review, presented objectively and impartially, and including nearly every contribution to our knowledge of wheat constituents that has been reported in the literature up to 1943, starting with Becarri's famous "gluten washing" experiment which was announced in 1728 and published in 1745.

As anticipated by the author, in the introduction, some readers will consider that there is too much detail in the recording of numerous items which are of historical interest only, and are obviously of no practical value in the light of current knowledge. In this reviewer's opinion, however, this is a minor matter. Of far greater importance is the painstaking thoroughness with which the entire world literature has been covered. All cereal technologists will gratefully acknowledge the real service that Dr. Bailey has rendered in making such a comprehensive and unique compilation conveniently available to them.

There are 16 chapters, covering the known and reported constituents of wheat. Seven chapters deal with the nitrogen compounds, and three with the carbohydrates. The remaining six are concerned, respectively, with the lipids, minerals, halogens (together with sulfur and selenium), acidity, pigments, and vitamins.

Some readers will question the propriety of referring to the use of the Kjeldahl method as "Kjeldahling." If this becomes standard practice we may expect eventually to see statements to the effect that a substance was "Sorensened," "Fischered," or "Van Slyked."

Literature citations are complete. There is an author index, and the subject index is adequate. A number of typographical errors are noted, but this is quite excusable under existing circumstances.

Every cereal chemist will wish to own a copy of this excellent book, which is the first and only one of its type in existence. The introduction contains a hint that Dr. Bailey is planning another volume, which will deal with the "dynamic biochemistry" of wheat and will consider enzymes, bread making, wheat processing, etc. Such a volume would be enthusiastically welcomed, and it is hoped that it will eventually make its appearance.

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## GELATINIZATION STUDIES UPON WHEAT AND OTHER STARCHES WITH THE AMYLOGRAPH<sup>1</sup>

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(Presented at the Annual Meeting, May 1942; received for publication January 19, 1944)

In recent years, considerable attention has been given to the development of convenient tests for the detection of excess amylase activity in relation to its undesirable effects on the crumb characteristics of baked goods. Kozmin (1933) and Molin (1932, 1934) were apparently the first to point out that excessive enzymic degradation of starch during baking is responsible for the production of bread with a moist sticky crumb of poor eating quality because of the decreased ability to bind water set free by coagulation of the gluten proteins.

The control of alpha-amylase activity appears to be of particular importance in the manufacture of breads made largely from rye flours, and Brabender (1937) described a recording viscosimeter for evaluating the baking quality of these flours. This apparatus, known as the amylograph, provides a continuous automatic record of the changes in viscosity of a flour-water suspension as the temperature is increased at a uniform rate. The increase in viscosity which takes place upon gelatinization of the starch is opposed by the liquefying action of the amylase present and the height of the curve at maximum viscosity is considered an index of amylase activity (Brabender, 1937; Brabender, Mueller, and Köster, 1937). The amylograph has been applied to flour quality studies by Schmidt and Scholz (1938); Brabender, Mueller, and Heide (1938); and Scholz (1940). Other torsion-type instruments have been developed which, like the amylograph, permit a study of the relative viscosity changes which occur in starch suspensions with increases in temperature (Caesar, 1932; Caesar and Moore, 1935; Radley, 1940; Barham, Wagoner, and Reed, 1942). The course of starch gelatinization may also be followed by measuring the increase in light transmission of starch suspensions upon heating (Cook and Axtmayer, 1937).

<sup>1</sup> Paper No. 2178, Scientific Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by Charles A. Anker to the faculty of the graduate school in partial fulfillment of the requirements for the degree of Master of Science, December, 1942.



The recent studies of Hollenbeck and Blish (1941) justify the use of starch liquefaction as a measure of the dextrinization of starch by alpha-amylase. In testing wheat or rye flours, however, several variables, such as starch content, inherent starch characteristics, extent of mechanical injury of the starch, and pH, may materially influence the paste viscosity in addition to differences in the extent of liquefaction which results from variations in alpha-amylase activity. Although there may be definite limitations in the general use of the amylograph as an index of the relative alpha-amylase activity of flours, it would appear to provide a convenient means for investigating differences in the pasting properties of starches from various sources; also for studying the relative resistance of different starches to alpha-amylase and the effects of different processing treatments used in the starch industry.

The studies reported in this paper represent preliminary investigations designed to determine the possible utility of the amylograph in flour and starch technology. In addition to experiments related to the technique of operation, the amylograph was employed in studies of the relative gelatinization characteristics of wheat, corn, and potato starch at various concentrations, of the effect of storage on the paste viscosity of wheat starch, and of various agents on pasting properties.

### The Amylograph and Its Operation

*Description and Operation.* The amylograph is a torsion viscosimeter which automatically records the resistance to shear offered by a flour or starch suspension as the temperature of the suspension is increased at a constant rate of approximately 1.5°C per min. The cylindrical, tinned-brass bowl (operating capacity 500 ml) in which the suspension is placed contains eight fixed, vertical pins and is rotated at the rate of 75 rpm in an electrically heated air bath by means of a synchronous motor which also operates the kymograph and the device for controlling the rate of temperature increase. The customary viscosimeter bob is replaced by seven metal pins attached to a circular metal disc around a central shaft, which is connected at its upper end to a coiled-wire spring; this, in turn, is fastened to the lever and pen of the kymograph. The rotation of the bowl forcing the suspension past the pins exerts a stirring effect and the frictional resistance causes the free-moving pins to rotate on their central axis against the resistance of the coil-spring. The extent of rotation is recorded by the kymograph in arbitrary units ranging from zero to 1,000 with rulings at 20-unit intervals; time rulings are provided for 1-min intervals.

The heater circuit is controlled by a contact mercury thermometer which functions as an ingenious thermoregulator. Provision is made for elevating the upper contact wire of the thermometer by means of the

synchronous motor, at a rate which requires a temperature rise of about  $1.5^{\circ}\text{C}$  per min to complete the circuit between the two contacts. The movement of the contact may be arrested at any desired temperature by means of a clutch which disengages the driving mechanism. A crank is provided for the manual setting of the contact at any desired temperature. The head of the amylograph, which supports the thermoregulator and shaft carrying the movable pins, may be raised by a lever to permit these parts to be swung out of position for convenient filling and removal of the bowl.

The operation of the amylograph has been described by Brabender, Mueller, and Köster (1937) and is quite simple. With starches, a convenient weight (usually 40–50 g) is placed in a 500 ml Erlenmeyer flask and 250 ml of distilled water at  $25^{\circ}\text{C}$  added; the flask is then stoppered and shaken to form a smooth suspension which is poured into the amylograph bowl. Wheat flours are mixed with the water in a small bowl by means of an egg beater in order to avoid lumping. An additional 200 ml of distilled water at  $25^{\circ}\text{C}$  is employed to rinse out the Erlenmeyer flask or bowl, and the rinsings are added to the suspension. The head of the amylograph is swung into position and the thermometer and movable pins are lowered into place. The upper contact of the thermometer is set at  $25^{\circ}\text{C}$ , the kymograph adjusted, and the control switch turned on. The temperature of the suspension is normally allowed to increase to  $95^{\circ}\text{C}$  when it is held constant. The apparatus is usually operated for a total elapsed time of 60 min.

*Relation of Response to Shearing Stress.* It was necessary, first, to ascertain whether the kymograph readings bore a linear relation to the force acting against the coil-spring of the instrument. An approximate test of the linearity of response was made in the following manner. The amylograph was completely assembled and the bowl left empty. A short lever was attached to the cover of the bowl and to this a strong cord was tied which passed over a small pulley and had an aluminum weighing pan fastened to the free end. The pulley was so arranged that the force applied to the cord was exerted at right angles to the lever at an amylograph reading of 500 units, that is, at the midpoint of the total angle (approximately  $60^{\circ}$ ) described by the lever in covering the entire range of the instrument. With the writing arm adjusted to zero, weights ranging from 20 to 230 g in 10-g increments were placed on the pan and the amylograph units recorded. The data, given in Table I, deviate only slightly from a linear relationship. Some deviation would be expected since the applied force was not uniformly exerted at right angles to the lever. Also, the applied load tended to pull the shaft to one side, thereby increasing the friction against the bearings as the load became greater. It may be concluded that the

amylograph readings are essentially directly proportional to the resistance to shear of the medium under test.

*Form and Evaluation of the Amylograph Curve.* For convenience, the resistance to shear, as measured in arbitrary units by the amylograph, will be called "viscosity" throughout this paper. It is recog-

TABLE I  
RELATION BETWEEN AMYLOGRAPH READING AND APPLIED LOAD<sup>1</sup>

Load	Amylograph reading	Load	Amylograph reading	Load	Amylograph reading
g	B. U.	g	B. U.	g	B. U.
20	90	90	465	160	775
30	155	100	510	170	810
40	215	110	560	180	850
50	250	120	600	190	890
60	315	130	650	200	930
70	365	140	690	210	960
80	410	150	730	220	990

<sup>1</sup> Recorded values apply to weights added and do not include the weight of the cord and pan.

nized that the resistance to shear or apparent viscosity depends upon several factors, such as the extent of aggregation of the granules, the extent of swelling (which not only influences viscosity by altering the volume relation between disperse phase and dispersion medium, but also by its influence on the degree to which the granules may be deformed under pressure), and the extent of granule disintegration or rupture, which not only changes the volume relation between disperse phase and dispersion medium but also the composition of the latter. Above certain limiting or critical concentrations which depend on such factors as the kind of starch, the extent to which it has been modified by pretreatment, and the pasting conditions, the viscosity of starch pastes is dependent upon the rate of shear, that is, such pastes exhibit anomalous or structural viscosity. These limiting concentrations are rather low for unmodified starch pastes: corn starch suspensions, for example, show anomalous viscosity at concentrations above about 2% when pasted at 90°C (Brimhall and Hixon, 1942). It is well known that starch pastes exhibit thixotropy, that is, they have gel properties when quiescent, become more fluid on the application of a shearing force, and again behave as gels when allowed to return to the quiescent state. Brimhall and Hixon (1942) state that the amylograph and consistometer "give results in which structural viscosity is subordinated to the resistance of the granules to crushing and the thixotropic characteristics of the pastes."

Several workers have established that when an aqueous starch suspension is gradually heated, the granules lose their characteristic birefringence before appreciable swelling occurs. Upon further heat-

ing, swelling becomes pronounced with a resulting increase in viscosity; in the absence of mechanical action, relatively little granule disintegration and solubilization of the starch takes place. Gallay (1936) and Gallay and Bell (1936) have concluded that the viscosity of a starch paste that has not undergone any severe pretreatment depends on the volume relation between disperse phase and dispersion medium and on

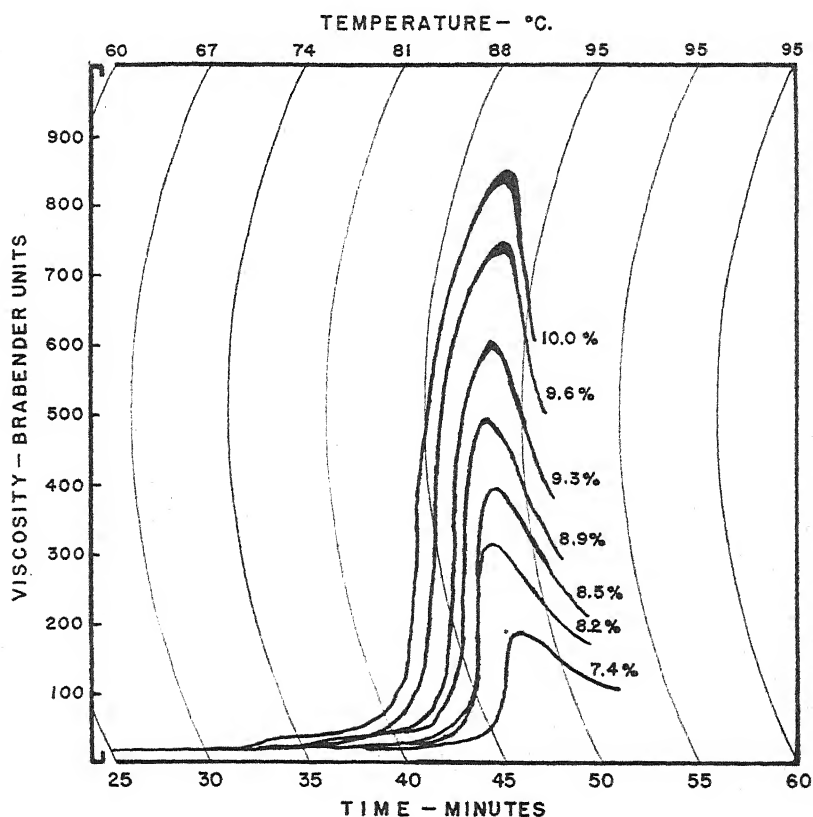


Fig. 1. Effect of wheat starch concentration on amylograph curve characteristics.

the deformability of the swollen granules. From studies of the viscosity changes which occur in corn and potato starch pastes on heating and stirring, Katz (1938) concluded that the heat gelatinization curve is a result of two opposing factors: one, the progressive swelling and hydration of the starch granule (which increases viscosity), and the other, the breakdown of the vesicle walls (which decreases viscosity). Schoch (1941) has pointed out that the viscosity of a boiled starch paste must be due largely to the presence of swollen aggregates or

fragments of granule structure since the viscosity markedly decreases upon autoclaving or violent mechanical agitation.

Representative amylograph curves made with commercial wheat starch suspensions of varying concentration are shown in Figure 1. The particular amylograph used in this study provided a temperature increase of  $1.39^{\circ}\text{C}$  per min. The general form of the gelatinization curve is similar to that obtained by Caesar (1932) with the consistometer, and by Barham, Wagoner, and Reed (1942) with their rotating cylinder viscosimeter. As pointed out by Caesar, the initial flat portion of the curve represents the period where any swelling is insufficient to register an increase in viscosity with the instrument; as the temperature is raised swelling becomes more and more pronounced, with a resultant increase in viscosity. Granule disintegration is not an important factor affecting the viscosity until swelling has progressed to the point where the granules become rather closely packed. As the packing becomes closer, the internal shearing stress increases with a concomitant increase in the extent of granule rupture. At the peak viscosity, swelling has nearly reached a limiting value and the influence of the small remaining increase in swelling is counterbalanced by any viscosity-decreasing factors which are operative. From this point on, the latter predominate, and further stirring and heating result in a decrease in viscosity. Although granule disorganization is the commonly accepted explanation of the sharp recession of the pasting curve, an increase in the permeability of the swollen granules may play a part in this phenomenon.

As the starch concentration increases, there is an appreciable decrease in the temperature at which the viscosity shows a measurable change, a marked increase in maximum viscosity, and a slight decrease in the temperature of the paste at which the peak viscosity is registered; moreover, the peak viscosity is more abrupt, and the subsequent decrease in viscosity is more rapid. Caesar (1932) and Barham, Wagoner, and Reed (1942) have reported similar findings. As the starch concentration is increased, a given degree of swelling will have a greater effect on the viscosity of the suspensions; consequently, a lower temperature and less swelling will be necessary to bring about a viscosity increase sufficient to be recorded by the amylograph. It is noteworthy that the amylograph is relatively insensitive to any swelling which occurs at temperatures below about  $71^{\circ}\text{C}$ . As pointed out by Caesar (1932), the lower paste temperature and the increase in slope of the down-gradient portion of the curve, as the starch concentration is increased, may be explained as being the result of greater granule rupture due to closer packing of the swollen granules.

As it is impractical to reproduce large numbers of gelatinization

curves, it is necessary to adopt some simple means of presenting their most significant characteristics in tabular form. For this purpose, three measurements can be readily taken from the curves: (1) the temperature at which the first perceptible increase in viscosity occurs; this has been conveniently called the temperature of transition by Cook and Axtmayer (1937); (2) the maximum viscosity; and (3) the temperature at which maximum viscosity is attained.

*Precision of Amylograph Values.* As an index of the precision of the amylograph, an analysis was made of the data for 69 sets of duplicate values obtained in connection with the various studies reported in this paper. The results were as follows:

Variable	Mean value	Mean difference between duplicates	Standard error (single determination)
Temperature of transition, °C	74.4	0.64	0.95
Paste temperature at maximum viscosity, °C	88.5	0.20	0.30
Maximum viscosity, Brabender units	59.5	6.2	9.3

Because of the gradual initial increase in viscosity, the error in estimating the temperature of transition from the curve is much higher than that involved in estimating the paste temperature at maximum viscosity. Considering the magnitude of the values, the replicate error for maximum viscosity is very satisfactory, especially since the kymograph paper is only ruled to 20 units; on the basis of the mean value of this variable, the error is 1.6%.

*Effect of Variations in Technique.* Since the viscosities of the wheat starch suspensions shown in Figure 1 did not increase until temperatures of from 71° to 83°C (depending upon the concentration) were reached, after 33 min or more of heating, it appeared that the test might be speeded up by employing a starting temperature just below the temperature of transition. The influence of starting temperature on curve characteristics was studied by preparing, in duplicate, five suspensions (containing 9.1% of wheat starch) at temperatures varying between 25° and 65°C; gelatinization curves were made with the contact thermometer set initially at the respective temperatures employed in preparing each suspension. The mean results, summarized in Table II, show a marked increase in maximum viscosity with starting temperatures exceeding 45°C. These results emphasize the importance of maintaining a uniform rate of heating throughout the entire course of the swelling and gelatinization process if consistent results are to be obtained. In fact, uniformity of technique in preparing the suspensions is also important. Allowing the prepared suspensions to stand

TABLE II  
EFFECT OF STARTING TEMPERATURE ON THE AMYLOGRAPH CURVES FOR 9.1%  
SUSPENSIONS OF COMMERCIAL WHEAT STARCH

Starting temperature	Temperature of transition	Paste temperature at max. viscosity	Maximum viscosity
°C	°C	°C	B. U.
25	68.8	90.5	658
35	67.0	91.0	690
45	72.9	90.8	692
55	65.6	90.8	778
65	73.3	91.4	816

for 15 min before commencing a test was found to increase slightly the temperature of transition and the maximum viscosity.

In subsequent experiments, the curves were started at 25°C immediately after the suspensions were prepared. Employing the standard technique of heating to 95°C and then holding the temperature constant at this value for a total elapsed time of 60 min, wheat starch suspensions (9.1% starch) lost 23 to 24 g of water by evaporation.

#### Gelatinization Characteristics of Corn, Wheat, and Potato Starch

To secure an index of the difference in amylograph curve characteristics for unmodified commercial corn, wheat, and potato starch, curves were made with these starches at suitable concentrations to give maximum paste viscosities which fell within 700 and 800 Brabender units. The results of mean determinations are given in Table III.

TABLE III  
GELATINIZATION CHARACTERISTICS OF POTATO, CORN, AND WHEAT STARCH

Starch	Starch concentration	Temperature of transition	Paste temperature at max. viscosity	Maximum viscosity
	%	°C	°C	B. U.
Potato	5.0	64.6	88.9	720
Corn	7.0	73.7	90.0	745
Wheat	10.0	70.2	91.2	830

The potato starch gave a viscosity increase at a much lower temperature than the corn and wheat starch and the maximum paste viscosity was reached at a slightly lower temperature. The viscosity of the potato starch rose very rapidly after swelling began and exhibited a much broader maximum and lower rate of decrease than that of corn or wheat starch.

To secure a comparison of the relative paste viscosities of the three starches, amylograph curves were made with each starch at a series of concentrations. The results are given in Table IV. As shown in Figure 2, when the logarithms of the maximum viscosities are plotted

TABLE IV  
EFFECT OF CONCENTRATION ON MAXIMUM VISCOSITY OF POTATO, CORN,  
AND WHEAT STARCH

Potato starch		Corn starch		Wheat starch	
Starch concentration	Maximum viscosity	Starch concentration	Maximum viscosity	Starch concentration	Maximum viscosity
%	B. U.	%	B. U.	%	B. U.
2.17	135	5.86	220	8.54	230
2.60	210	6.64	320	9.27	350
3.02	290	7.02	405	10.00	485
3.43	390	7.22	420	10.71	570
3.85	510	7.41	470	11.42	710
4.05	590	7.79	545	12.11	875
4.26	660	8.16	610		
4.66	825	8.54	720		
		8.91	810		

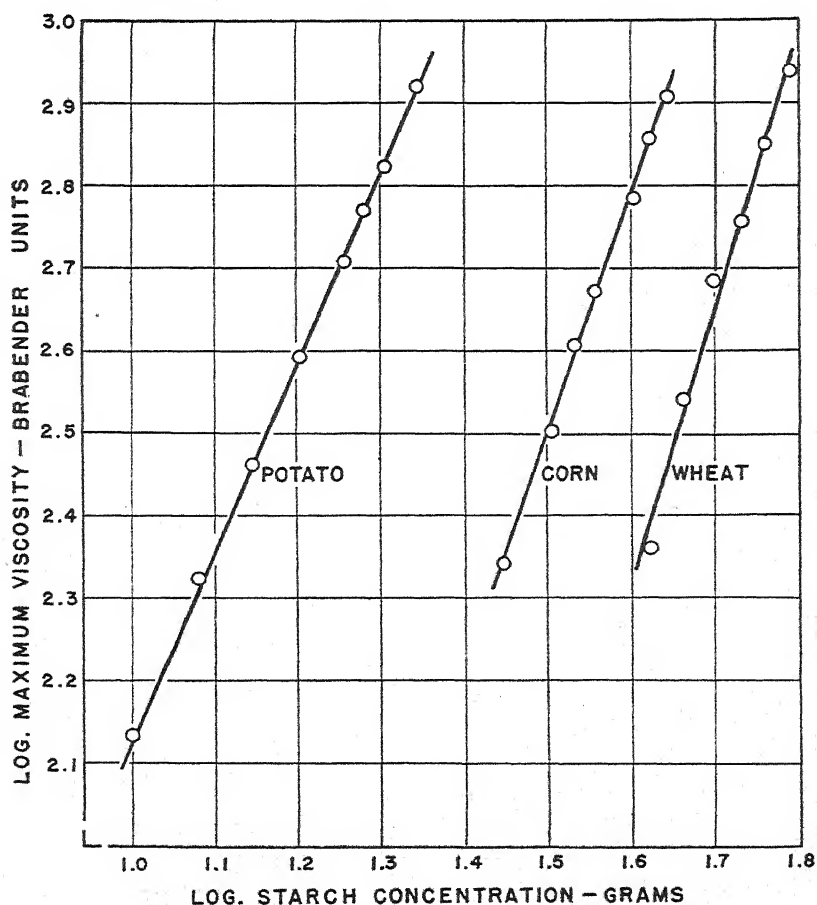


Fig. 2. Relation between concentration and maximum paste viscosity of potato, corn, and wheat starch.



against the logarithms of starch concentration a straight line results for each starch. These data show the wide differences in paste viscosity of the three starches at equivalent concentrations.

### Mathematical Relation between Starch Concentration and Paste Viscosity

Brimhall and Hixon (1942) have recently reviewed the various equations proposed for expressing the relation between viscosity and the volume or the concentration of the starch granules. In studies with a series of wheat starches, Rask and Alsberg (1924) found that by plotting the logarithms of the viscosity of the pastes, as determined at 90°C with a Stormer viscosimeter, against starch concentration (2.8%–5.8%), a straight line resulted. Brimhall and Hixon, in determining the hot paste viscosities of unmodified corn starches, in a capillary viscosimeter, found that the logarithmic relation of Rask and Alsberg held only over certain ranges of concentration and pressure.

The linear relation between the logarithms of maximum viscosity and logarithms of starch concentration noted in the present work was found to hold with unmodified potato, corn, and wheat starch over all ranges in concentration investigated. This relation is represented by the formula  $y = kx^n$ . Should further investigation establish that it holds generally for various native and modified starches, it would be of considerable practical significance. By making a series of viscosity determinations over a range of concentrations, regression equations could be established for each starch type, from which the paste viscosity corresponding to any desired concentration could be computed, or vice versa.

An explanation<sup>2</sup> of the observed relationship between maximum viscosity and initial starch concentration may be reached if it is assumed:

(1) that during the gelatinization process, the ungelatinized granules A form highly swollen gelatinized granules B which are then eventually ruptured as a result of shearing action, thereby losing most of their incorporated water and forming relatively nonhydrated disintegrated granules C; (2) that the viscosity increment due to A and to C is small or insignificant as compared with the viscosity increment due to B; (3) that the viscosity-concentration relationship with respect to B is approximately described by the Arrhenius equation: that is  $\log \eta_r = K[B]$  (where  $[B]$  denotes the concentration of B); (4) that  $\eta_s \gg \eta_o$  = where  $\eta_s$  = viscosity of solution and  $\eta_o$  = viscosity of solvent. The relative viscosity  $\eta_r$  would then be approximately proportional to  $\eta_s$ .

<sup>2</sup> The authors are indebted to D. R. Briggs, Division of Agricultural Biochemistry, University of Minnesota, for the explanation referred to.

and the value  $\eta_s$  could be substituted for  $\eta_r$  in the Arrhenius equation; that is,  $\log \eta_s = K[B]$ ; (5) that the process  $A \rightarrow B$ , involving the taking up of water by the granule, is a first-order process. At the end of a given time, the amount of B present would then be directly proportional to the initial amount of A present. This process could be expected to be of the first order as long as there was sufficient water present so that no competition occurred between granules for the water; (6) that the process  $B \rightarrow C$ , involving the rupture or disintegration of the gelatinized granules, is a second- or higher-order process. This would be expected since the rate of disintegration should be a function of the shearing action on the swollen granules, and the intensity of this shearing action is a function of the viscosity, which in turn is a function of the concentration of B.

On the basis of these assumptions, the amount of B formed in a given time after the process began would be proportional to the initial concentration of A; however, as the initial concentration of A is increased, the maximum amount of B would be reached in a shorter time, and the value of the maximum attained for B would vary logarithmically with the initial concentration of A (or to the amount of B formed in each comparable unit of time after the process  $A \rightarrow B$  is initiated):

$$\text{thus,} \quad [B]_{\max.} = K \log [A]_{\text{initial}}$$

$$\text{and} \quad \log \eta_{s\max.} = K'[B]_{\max.}$$

$$\text{then} \quad \log \eta_{s\max.} = K'K \log [A]_{\text{initial}};$$

this is the observed relationship. The amylograph curves also show a slight displacement of the maxima toward lower time values as the initial starch concentration is increased (see Fig. 1). This is not marked because of the very short time interval which is required for the entire process:  $A \rightarrow B \rightarrow C$ .

### Effect of Various Agents on the Pasting Properties of Starch

#### *Effect of Flour Proteins upon Amylograph Curves for Wheat Starch.*

In interpreting peak viscosities obtained with suspensions of wheat flours as a measure of flour amylase activity, it must be assumed that the viscosities are not materially influenced by other variables. The marked influence of starch concentration on maximum viscosity indicates that this variable would have to be carefully controlled. In wheat flours, however, a decrease in starch is accompanied by an increase in protein; in flours of equivalent extraction the sum of these two constituents may be regarded as being approximately equal. It was therefore of interest to determine the effect of complementary variations in starch and protein content on maximum viscosity.

Gluten was washed from an undiastated hard red spring wheat flour, dried in thin layers at 25°C under vacuum, and finely ground; it contained 82% of protein (dry matter basis). Two series of amylograph curves were made. One series comprised mixtures of this gluten and commercial wheat starch in which the protein content was varied from 6 to 16% in 2% increments; the other series was made with starch alone, employing the respective quantities present in the various starch-gluten mixtures. In making the curves for the mixtures, the gluten was hydrated for 2 hr in a 100-ml portion of the water used to suspend the starch.

Since flour amylases are, in part, absorbed on, or occluded in, the gluten fraction, the amylase activity of the gluten-starch mixtures would be expected to increase with increasing gluten content. The diastatic activities of the starch, and of starch-gluten mixtures containing 6 and 16% protein, were determined as outlined in Cereal Laboratory Methods (4th ed., 1941); the respective mean values were 8, 16, and 18 units. It may be assumed that these relatively small differences in diastatic activity would not materially influence the gelatinization characteristics, especially since they may be ascribed chiefly to beta-amylase activity.

TABLE V  
EFFECT OF STARCH CONCENTRATION AND ADDED GLUTEN  
ON MAXIMUM PASTE VISCOSITY

Protein added as gluten	Maximum paste viscosity <sup>1</sup>	
	Starch-gluten mixture	Starch
%	B. U.	B. U.
0	952	952
6	760	728
8	702	640
10	630	558
12	600	505
14	558	452
16	500	390

<sup>1</sup> The total dry weight of starch and protein was maintained at 46 g in 450 ml of distilled water. The values recorded in the starch column were obtained by gelatinizing starch suspensions which contained the same concentration of starch as was present in the starch-gluten mixtures.

The mean maximum viscosities recorded in Table V show, as anticipated, that the substitution of gluten proteins for an equivalent weight of starch decreases the maximum paste viscosity. At equal starch concentrations, however, the presence of gluten increases viscosity. These results imply that, in studying alpha-amylase activity of flours with the amylograph, the effect of variations in protein and starch content cannot be eliminated by weighing the samples on a constant-protein, constant-starch, or constant-protein-plus-starch basis.

*Effect of pH on Amylograph Curves for Wheat Starch.* The effect of pH on the amylograph curve for commercial wheat starch was investigated over the normal range for fermenting doughs (pH 5.2–6.7). Two buffer mixtures were employed, namely, 0.05*M* bimalate buffers (prepared according to Temple, 1929), which covered the entire range desired, and 0.05*M* citrate buffers (prepared according to Kolthoff and Vleeschouwer, 1926), which covered the pH range of 5.2–6.0. The curves were made with 45 g (dry basis) of commercial wheat starch and 450 ml of the respective buffer solutions. A glass electrode was used in determining the pH of the suspensions. The mean results of duplicate determinations are summarized in Table VI.

TABLE VI  
EFFECT OF PH AND BUFFER COMPOSITION ON THE AMYLOGRAPH  
CURVE CHARACTERISTICS OF COMMERCIAL WHEAT STARCH<sup>1</sup>

pH of suspension	Temperature of transition	Paste temperature at max. viscosity	Maximum viscosity
	°C	°C	B. U.
Control			
4.30	82.7	93.2	880
Bimalate buffer mixture			
5.26	79.2	92.8	932
5.72	79.2	93.3	870
6.08	79.2	93.8	836
6.42	79.9	94.2	780
6.71	79.2	94.9	760
Citrate buffer mixture			
5.32	81.3	93.8	910
5.79	82.0	94.5	830
6.13	79.2	95.2	750

<sup>1</sup> Suspensions contained 9.1% starch.

The maximum paste viscosity for each buffer, respectively, decreased in linear fashion with an increase in pH; the decrease was greater for the citrate than for the bimalate buffer. However, the control sample, which was the lowest in pH, gave a greater peak viscosity than several of the suspensions which were buffered at higher pH values. This, together with the differences in the effect of the two buffer solutions, leads to the suggestion that the results may be due to the effects of ion adsorption on the permeability of the granule.

*Effect of Alpha-Amylase on the Gelatinization Curve for Various Wheat Starches.* The effect of alpha-amylase activity on the form of the amylograph curve for commercial wheat starch was followed over a diastatic activity range of 17 to 600 maltose units, as determined by the regular A. A. C. C. procedure for wheat flour (Cereal Laboratory Methods, 4th ed., 1941). Takadiastase (undiluted; Parke Davis and Co.) was used as a convenient means of increasing the alpha-amylase

activity of the starch. The quantity of takadiastase varied between 0 and 2% of the weight of the starch and was dispersed in the water used in preparing the starch suspensions. To secure full advantage of the amylograph scale range, the curves were made with 60 g of starch (dry basis) and 450 ml of distilled water or takadiastase dis-

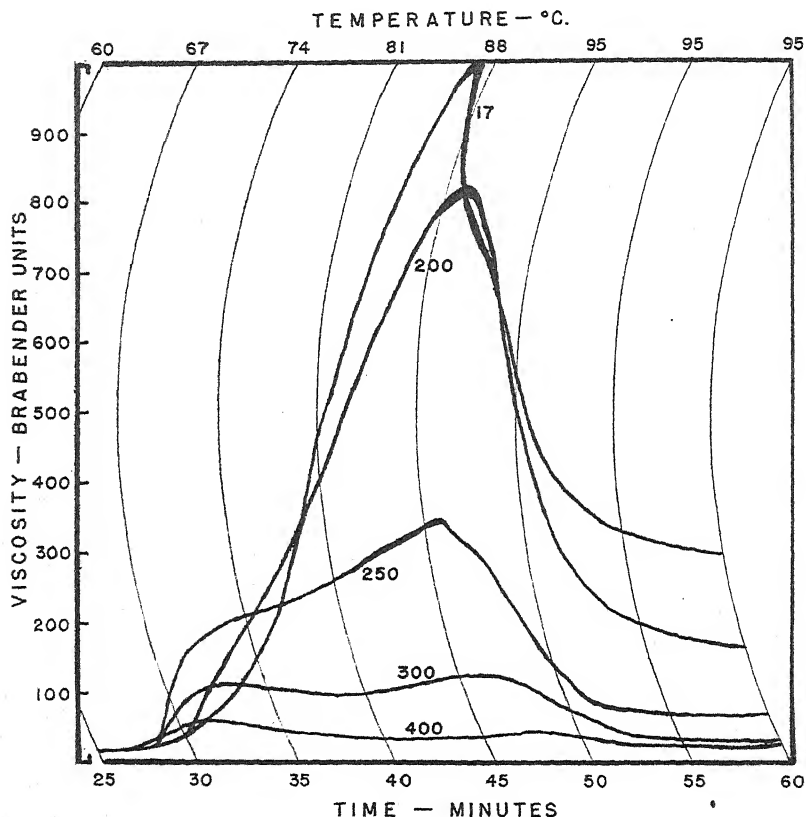


Fig. 3. Amylograph curves for commercial wheat starch brought to diastatic activities of 17, 200, 250, 300, and 400 maltose units (dry basis) by addition of takadiastase. The starch suspensions contained 11.8% starch.

persion. The pH of the various suspensions was 4.3. The maltose content of the gelatinized pastes was determined by the ferricyanide procedure employed in determining diastatic activity, immediately following the 60-min gelatinization period.

Figure 3 shows representative amylograph curves for commercial wheat starches brought to diastatic activity levels of 17 to 400 maltose units. Mean curve readings for the entire series, together with the quantities of maltose produced during gelatinization, are given in Table VII.

These data show the marked liquefying action of alpha-amylase. As measured by the diastatic activity of the starch-takadiastase mixtures, the effect of alpha-amylase on paste viscosity is curvilinear. As the maximum viscosity decreased, the peaks became less sharp than in the instance of lower peak viscosities due to a decrease in starch concentration (Fig. 1). The curves for wheat starch with diastatic activities of 300 and 400 units exhibited two ill-defined peak viscosities at widely different paste temperatures.

TABLE VII

EFFECT OF ADDED TAKADIASTASE ON AMYLOGRAPH CURVE CHARACTERISTICS FOR COMMERCIAL WHEAT STARCH, AND ON MALTOSE PRODUCTION DURING PASTING

Diastatic activity of starch	Amylograph curve characteristics			Maltose produced <sup>1</sup>
	Temperature of transition	Paste temp. at max. viscosity	Maximum viscosity	
<i>maltose units— mg/10 g</i>	<i>°C</i>	<i>°C</i>	<i>B. U.</i>	<i>mg/10 g</i>
17	64.6	86.8	1,000	108
100	64.6	87.8	988	133
200	64.6	88.8	798	633
250	63.9	88.2	410	1,053
300 <sup>2</sup>	63.9	90.0	133	2,400
400 <sup>2</sup>	62.8	68.1	62	4,933
500	63.9	67.4	36	8,600
600	64.6	67.4	31	9,600

<sup>1</sup> Maltose expressed as mg of maltose per 10 g of starch produced during the 60 min required for amylograph test.

<sup>2</sup> Two peak viscosities were observed; the paste temperatures and maximum viscosities given are for the highest peak viscosities.

Increasing alpha-amylase activity had no significant influence on the temperature of transition but the paste temperature at maximum viscosity showed a sudden decrease when the diastatic activity of the starch was increased from 300 to 400 maltose units.

The liquefying effect of alpha-amylase next was investigated with wheat starches prepared in the laboratory from five commercially milled flours: a durum fancy patent, southwestern winter wheat patent, hard red spring wheat patent, soft wheat patent, and a Minnesota winter wheat patent. Each flour was mixed to a stiff dough with water; after standing in water at 15°C for one hr, the gluten was washed out with tap water and the starch recovered from the wash water by centrifuging. After washing three times with distilled water, the starch was dried at room temperature in a current of air. Three levels of takadiastase (0.042, 0.210, and 0.525%), which gave diastatic activity values of 100, 200, and 300 maltose units with the commercial wheat starch previously studied, were added to each starch and the diastatic activities then determined. Amylograph curves were made

in duplicate employing 45 g of starch and the selected levels of takadiastase with 450 ml of water.

The results are recorded in Table VIII; the relation between diastatic activity of the starch preparations and the maximum viscosity of the pastes is shown in Figure 4. The wide differences in apparent

TABLE VIII  
EFFECT OF ADDITIONS OF TAKADIASTASE UPON THE DIASTATIC ACTIVITY AND  
AMYLOGRAPH CURVE CHARACTERISTICS OF DIFFERENT WHEAT STARCHES  
(Suspensions contained 9.1% starch)

Takadiastase added, %	Source of wheat starch				
	Durum wheat	Southwestern winter wheat	Hard red spring wheat	Minnesota winter wheat	Soft wheat
DIASTATIC ACTIVITY, MALTOSE UNITS					
nil	4	3	10	2	2
0.042	42	31	27	12	10
0.210	111	78	66	35	34
0.525	184	131	104	62	60
TEMPERATURE OF TRANSITION, °C					
nil	80.8	79.9	80.8	81.5	74.3
0.042	80.6	76.4	76.4	76.4	69.5
0.210	65.3	66.7	66.7	68.1	66.7
0.525	66.7	66.7	66.7	63.9	65.3
PASTE TEMPERATURE AT MAXIMUM VISCOSITY, °C					
nil	94.5	94.8	95.0	95.0	91.2
0.042	94.5	94.5	94.5	93.1	92.4
0.210	93.1	91.7	93.8	93.8	90.3
0.525	93.1	70.7	69.5	68.1	68.1
MAXIMUM VISCOSITY, BRABENDER UNITS					
nil	878	755	770	735	888
0.042	750	590	550	590	770
0.210	138	150	147	105	135
0.525	60	40	50	42	50

amylolytic susceptibility of the various starches, as indicated by the variations in diastatic activity for corresponding increments of added takadiastase, may, in part at least, be due to varying degrees of mechanical injury of the starches during milling (Malloch, 1929; Karacsonyi and Bailey, 1930; Sandstedt, Blish, Mecham, and Bode, 1937; Sandstedt, Jolitz, and Blish, 1939; Jones, 1940; and others). The temperatures of transition and paste temperatures at peak viscosity

of the undiastated starches from the different hard wheats did not differ significantly, but these values were appreciably lower for the starch from the soft wheat. Diastating the starches resulted in a lowering of both the temperature of transition and the paste temperatures at peak viscosity, particularly for the two highest levels of

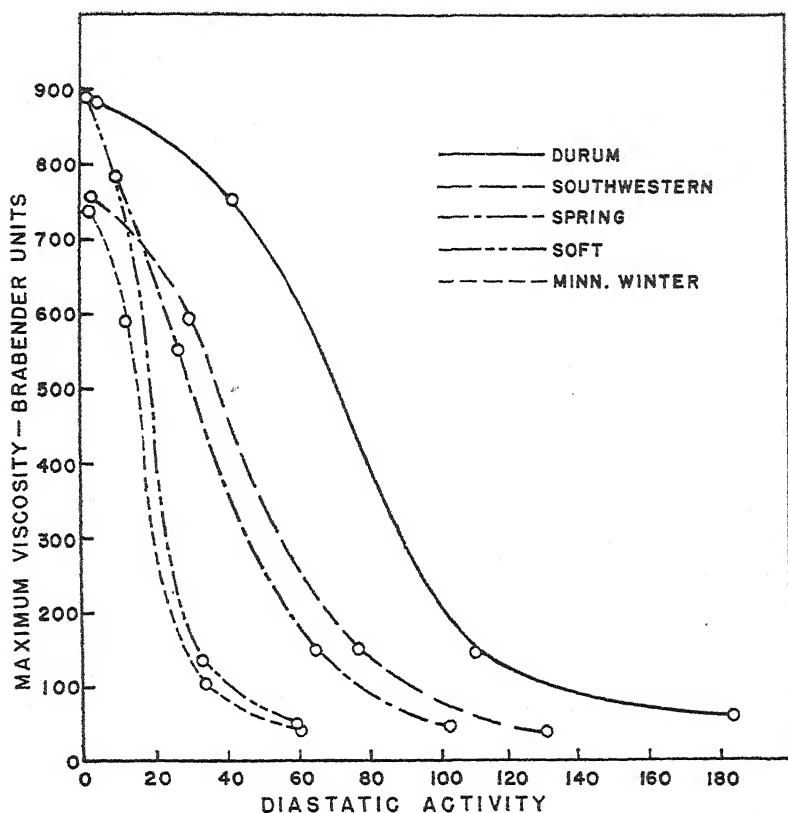


Fig. 4. Relation between diastatic activity and maximum paste viscosity for various wheat starch preparations. Diastatic activity was varied by additions of takadiastase to the starch. The starch suspensions contained 9.1% starch.

takadiastase. The maximum viscosity values for equal concentrations of the undiastated starches varied from 735 Brabender units for the Minnesota winter wheat starch to 888 units for the soft wheat starch. Increasing the diastatic activity markedly decreased maximum paste viscosity of each starch, but the relation between these variables is not strictly linear. Although the starches which showed the highest apparent amylolytic susceptibility suffered the greatest decrease in viscosity, the maximum viscosity corresponding to a given maltose value varied widely with the different starches. For example,



a peak viscosity of 400 Brabender units was given by starches varying in maltose value from approximately 20 to 85 units. Accordingly, maximum paste viscosity cannot be interpreted as a direct index of amylase activity unless something is known about the maximum viscosity to be expected from the starch (or flour) in the absence of amylase activity.

*Effect of Storage on Paste Viscosity of Commercial Wheat Starch.*  
A marked decrease was observed in the paste viscosity of commercial

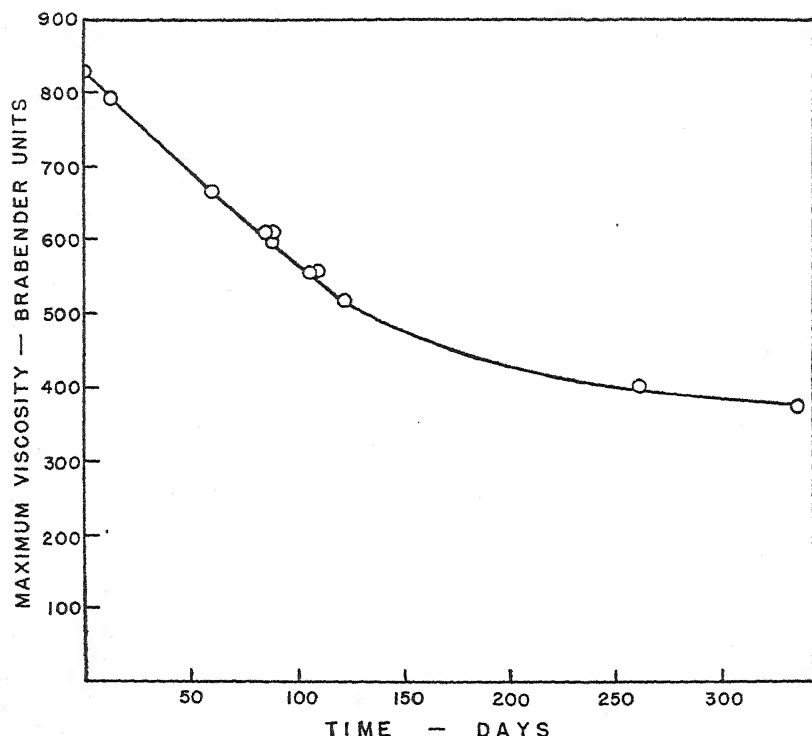


Fig. 5. Effect of time of storage of commercial wheat starch on maximum paste viscosity. Gelatinization tests were made with 9.1% suspensions.

wheat starch with time of storage. The starch contained 9.0% moisture (A. A. C. C. air-oven method) and was stored in glass bottles at laboratory temperature (22–25°C); the pH of a 10% suspension was 4.3, as determined with a glass electrode. Reitz, Gortner, and Carlson (1942) had previously noted the same phenomenon in the cold gelatinization behavior of air-dry wheat starch (10.6% moisture) which had been carefully prepared in the laboratory from Thatcher wheat.

The gelatinization behavior of the sample used in the present studies was followed in the amylograph at intervals over a period of

336 days, employing 50 g (dry basis) of starch and 450 ml of distilled water. As shown in Figure 5, the maximum viscosity dropped from an initial value of 830 B. U. to 370 B. U. after 336 days' storage. No consistent trends were observed in the temperature of transition or paste temperature at maximum viscosity. At the end of the storage period a portion of the starch was thoroughly washed with distilled water, centrifuged, and dried under vacuo at room temperature. This treatment was without any significant influence upon its gelatinization behavior. In view of these surprising results, time, and probably conditions of storage, are important factors to be taken into consideration in investigating the physicochemical properties of starches.

*Effect of Cold Gelatinizing Agents on the Heat Gelatinization of Wheat Starch.* Several reagents in aqueous solution cause starch to swell

TABLE IX  
EFFECT OF COLD GELATINIZING AGENTS UPON THE HEAT GELATINIZATION  
BEHAVIOR OF COMMERCIAL WHEAT STARCH

Gelatinizing reagent	Reagent concentration	Temperature of transition	Paste temp. at max. vis.	Maximum viscosity
	<i>M</i>	°C	°C	<i>B. U.</i>
Control		70.9	90.3	557
Sodium salicylate	0.01	66.7	89.6	625
	0.025	67.4	88.4	720
	0.05	66.0	86.3	797
	0.10	63.9	83.4	972
	0.10	67.4	88.2	838
Sodium thiocyanate	0.10	66.7	88.1	847
Potassium thiocyanate	0.10	67.4	88.2	837
Ammonium thiocyanate	0.10	66.7	89.6	785
Potassium iodide	0.10	67.4	90.0	576
Urea				

or gelatinize at ordinary temperatures. Following the qualitative studies of Reychler (1920), a number of workers, including Ostwald and Frenkel (1927), Katz (1933), and numerous other papers, Mangels and Bailey (1933, 1933a), and Mangels (1934, 1936), have made quantitative studies of the relative efficiency of various cold gelatinizing agents and have applied them in investigating starches from various sources. Caesar (1932), Wiegell (1934, 1936), and Cook and Axtmayer (1937) have carried out heat gelatinization experiments in the presence of various reagents in which the viscosity of the starch suspensions was followed as the temperature of the suspensions was increased at a constant rate.

In the present study, six representative gelatinizing agents were employed, namely: sodium salicylate, sodium thiocyanate, potassium thiocyanate, ammonium thiocyanate, potassium iodide, and urea. The first four compounds hydrolyze to form an alkaline solution, potas-

sium iodide is a neutral electrolyte, and urea represents an organic swelling agent which forms a basic solution. Suspensions made with 50 g of commercial wheat starch and 450 ml of 0.1*M* solutions of each of these reagents were gelatinized in the amylograph over the usual temperature range of 25° to 95°C. In addition, curves were made with distilled water and three lower concentrations of sodium salicylate. The results are summarized in Table IX.

As compared with heat gelatinization in water alone, the cold gelatinizing agents decreased the transition temperature and the paste temperature at maximum viscosity but markedly increased paste

TABLE X  
EFFECT OF SODIUM SALICYLATE ON THE HEAT GELATINIZATION OF WHEAT  
AND POTATO STARCH<sup>1</sup>

Sodium salicylate conc.	Temperature of transition		Paste temperature at max. viscosity		Maximum viscosity	
	Wheat	Potato	Wheat	Potato	Wheat	Potato
<i>M</i>	°C	°C	°C	°C	<i>B. U.</i>	<i>B. U.</i>
0	85.2	64.2	94.3	81.0	20	470
0.1	58.6	62.8	67.0	72.6	60	340
0.2	53.0	58.6	62.8	67.0	135	400
0.5	51.6	47.4	69.1	55.1	330	590
0.6		42.5		50.2		700
0.8	33.4	34.1	48.8	41.8	475	770
1.0	27.1	28.5	32.7	36.2	540	890
1.2	25.0	25.0	26.4	33.4	420	990
1.4		25.0		26.4		940

<sup>1</sup> Amylograph curves were made with 30 g of starch and 450 ml of water or sodium salicylate solution.

viscosity. Sodium salicylate was the most, and urea the least, effective agent. The relative efficiencies of the salicylate, thiocyanate, and iodide ions are in the same order as that found by Mangels and Bailey (1933), who observed a lyotropic anion effect in their cold gelatinization studies.

In view of the marked effect of sodium salicylate on the heat gelatinization curve for wheat starch, additional curves were made with 30 g (dry basis) of commercial wheat and potato starch in 450 ml of various concentrations of this salt.

The mean results, summarized in Table X, show the very marked effects of sodium salicylate in lowering the temperature at which starch swelling becomes perceptible, in lowering the paste temperature at maximum viscosity, and in increasing the maximum viscosity. The greatest relative increase in viscosity was obtained with wheat starch and the maximum was registered at a lower concentration of sodium salicylate than with potato starch.

The markedly higher peak viscosities obtainable with cold gelatinizing agents, as compared with heat gelatinization, is of theoretical interest. Gortner (1933) applied the Kunitz formula to secure an estimate of the relative volumes of the disperse phase in cold and hot gelatinization of starch; the calculations indicated that the volume of the swollen granules was very much greater for cold gelatinization. This observation is in accord with the markedly lower temperature and higher peak viscosities found in this study when cold gelatinizing agents are present. These agents must greatly increase the ability of the starch granules to swell without granule disintegration taking

TABLE XI  
EFFECT OF MODIFICATION ON CURVE CHARACTERISTICS FOR CORN STARCH

Sample No.	Corn starch	Temperature of transition		Paste temp. at max. viscosity		Maximum viscosity	
		Starch weight		Starch weight		Starch weight	
		35 g	50 g	35 g	50 g	30 g	50 g
		°C	°C	°C	°C	B. U.	B. U.
1	Unmodified	74.3		90.3		696	
2	Acid modified	74.3	70.9	90.0	85.8	138	422
3	Acid modified	73.0	70.9	87.6	82.4	120	405
4	Chlorinated	70.9	70.2	77.8	76.4	59	114
5	Chlorinated	68.1	66.4	73.0	72.5	54	85

place. In spite of extensive researches on the action of such agents, there is as yet no generally accepted theory which completely explains their behavior. Meyer (1942) has pointed out that starch grains have a limited capacity to swell in hot water and that such limited swelling is characteristic of chain polymers which are held together in large three-dimensional molecules by network secondary valence linkages which can be broken by chemically inert reagents. In starch, the swelling and solubilization of the amylose is limited by the lattice-like micelles formed by the amylopectin. If the secondary valence bonds acting between different parts of the amylopectin are broken, the micellar structure is opened up to form larger units thereby making it possible for the amylose molecules to take up more water and thus cause further swelling of the starch.

*Effect of Processing Treatment of Curve Characteristics for Corn Starch.* The characteristics of amylograph curves for five samples of commercial corn starch obtained from one source are summarized in Table XI. With one exception, two concentrations, namely 35 g and 50 g, of starch (dry basis) with 450 ml of distilled water, were employed. Starch No. 1 was a crude, unmodified or native starch; Nos. 2 and 3 were standard, acid-modified or thin-boiling starches representative of

the type used for warp-sizing in textile mills (No. 3 was the more highly modified); No. 4 was a chlorinated starch of the type used in sizing rayon, whereas No. 5 was a more highly modified chlorinated starch ordinarily used in the tub sizing of paper.

The relative curve characteristics for these starches are in line with the extent of their modification. Chlorination appreciably lowered the temperature of transition and the paste temperature at which maximum viscosity was attained, in addition to markedly decreasing the peak viscosity. The problem of evaluating starches prepared by various types and degrees of modification is a difficult one at present because they cover such a wide viscosity range that their relative viscosities cannot be satisfactorily determined by one method at one concentration. These limited trials suggest the possibility that relative values for the various types could be obtained in the amylograph because of the wide range of the instrument.

### Discussion

These survey experiments show that the amylograph provides a convenient means for carrying out technological studies of the pasting properties of starches and of the effects of various agents on gelatinization characteristics. Because of the wide range in the magnitude of the viscosities which can be recorded and the various temperatures at which it can be operated, it may well prove useful in investigating the viscous or plastic properties of substances other than starches and flours. From the fact that the machine is calibrated in arbitrary units, and the rates of shear and of temperature increase are fixed, it is more useful in technological studies than as a research instrument.

The marked liquefying action of alpha-amylase on the viscosity of starch paste favors the use of the amylograph as a convenient means of determining the alpha-amylase activity of wheat and rye flours. However, such variables as pH, starch content, protein content, inherent differences in starch characteristics, and in the extent of mechanical injury suffered by the starch during milling influence paste viscosity and hence would interfere with the interpretation of relative height of the amylograph curve as a direct index of the alpha-amylase activity of flours which differ widely in these characteristics. In mill-control work, these interfering factors would not come into full play; the mill mix for any particular type of flour represents a composite of certain restricted types of wheat and the protein content is controlled within rather narrow limits. How closely maximum paste viscosity would be correlated with alpha-amylase activity under such limited conditions must be determined by further experiments.

Two difficulties were encountered which indicate that some im-

provement in the design of the instrument would be desirable. In certain of the experiments with cold gelatinizing agents, clot formation occurred around the upper part of the fixed center pin in the amylograph bowl. The other difficulty arose with very viscous pastes at the end of the heating period, especially when maximum paste viscosity occurred around 95°C. Under these conditions the contents of the bowl occasionally boiled over. The stirring was inadequate to maintain a uniform temperature throughout these viscous pastes, which resulted in the overheating and boiling over of the starch paste in the proximity of the outer edge of the bowl. It is interesting to note that Barham *et al* (1942) observed irregularities in viscosity at high concentrations during the heating period, with their rotating cylinder viscosimeter; these were ascribed to clot formation resulting from nonuniformity of the pastes.

### Summary

Response of the amylograph to variations in the load applied to the viscosity-registering device was essentially linear. Wheat starch suspensions gelatinized in the amylograph from initial temperatures above 45°C gave markedly higher peak viscosities than corresponding suspensions gelatinized from lower initial temperatures. The precision of the measurement is satisfactory; for a series of 69 curves made in duplicate, the standard error (single determination) was 9.3 B. U.

With an increase in starch concentration, the temperature of transition and paste temperature at maximum viscosity decreased, the maximum paste viscosity increased, and the rate of decrease in viscosity after the maximum became greater. When the logarithm of the maximum viscosity was plotted against the logarithm of the starch concentration, a straight line resulted for all starches investigated (corn, potato, and wheat). This relation implies that starch swelling is a first-order process while granule disintegration is a second- or higher-order process.

Suspensions of wheat gluten and wheat starch gave higher paste viscosities than wheat starch suspensions of corresponding starch concentration.

Maximum paste viscosity of commercial wheat starch suspensions decreased in linear fashion with an increase in pH from 5.2 to 6.8. The decrease in viscosity was less with bimalate than with citrate buffers.

Maximum paste viscosity of commercial wheat starch suspensions was markedly lowered when the starch was brought to increased maltose values by the addition of takadiastase (employed as a source of alpha-amylase).

Wheat starches prepared from durum, hard red spring, hard red winter, and soft winter wheat flours gave maximum paste viscosities for 9% suspensions which varied from 735 B. U. for Minnesota hard winter to 888 B. U. for soft winter wheat starch. Corresponding additions of takadiastase to these starches resulted in wide differences in paste viscosity. The relative maximum viscosity values corresponded, in general, to the apparent amylolytic susceptibility of the starches as measured by maltose value. For any given paste viscosity, there was an appreciable range in the corresponding maltose value for the different starches.

Of several cold gelatinizing agents investigated with the amylograph, sodium salicylate was the most effective agent and urea the least effective. As compared with heat gelatinization in water alone, starch suspensions containing the more effective agents gave amylograph curves which were characterized by a lower transition temperature, lower paste temperature at maximum viscosity, and a markedly higher paste viscosity. Cold gelatinizing agents greatly increase the ability of the starch granules to swell without disintegration of the granules.

Wheat starch stored at 9.0% moisture at room temperature yielded suspensions of decreasing maximum paste viscosity with increased time of storage.

Marked differences were noted in the amylograph curve characteristics for native, acid-modified, and chlorinated corn starches.

The amylograph appears to provide a convenient means of investigating the pasting properties of starches from various sources, the relative resistance of different starches to amylases and other starch degrading agents, and the effects of different processing treatments.

Caution must be observed in interpreting the maximum paste viscosity of wheat- and rye-flour suspensions as an index of relative alpha-amylase activity of the flours because of the influence on paste viscosity of such variables as starch content, protein content, inherent differences between starches, extent of mechanical injury, and pH.

#### Acknowledgment

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## THE USE OF THE AMYLOGRAPH IN THE CEREAL LABORATORY

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Numerous researches have been conducted on the utility of viscosity determinations in evaluating cereal products. In these investigations, many of which gave conflicting results, viscosity was usually measured at a fixed temperature, but lately several workers have studied the viscosity of cereal products under flexible, controlled temperature conditions. There is now available a recording viscosimeter, the Amylograph, which greatly facilitates studies of this nature, since viscosity can be measured and recorded automatically, either at a selected fixed temperature or under uniformly rising temperature conditions. Anker and Geddes (1944)<sup>1</sup> have described this instru-

<sup>1</sup> Anker, C. A., and Geddes, W. F., (1944) Gelatinization studies upon wheat and other starches with the Amylograph. *Cereal Chem.* 21: 335-360.

ment and have reported the results of investigations in technique, viscosities of different starches upon gelatinization, and effects of alpha-amylase on the gelatinization curves of starches. They also report the literature on the subject.

The experiments reported in this paper were undertaken to determine the value of the Amylograph in evaluating the baking quality of rye, for measuring the susceptibility of different flours to alpha-amylase, and as an index of the heat treatment of soybeans. The effect of such variables as granulation, pH, and electrolytes on paste viscosity were also investigated.

### Experimental

*Relation between Maximum Viscosity and Baking Value of Rye Meals.* The baking quality of rye meals and flours is markedly influenced by their alpha-amylase activity. If this is too high, excessive liquefaction and dextrinization of the starch occurs, thereby lowering its ability to bind the water liberated by the denaturation of the proteins during baking. The bread from such flours and meals has a moist crumb with gummy characteristics. On the other hand, insufficient alpha-amylase is characterized by a dry, brittle crumb. Since these crumb properties are associated with the liquefying and dextrinifying action of alpha-amylase, a determination of the viscosity of gelatinized suspensions should provide a convenient means of evaluating rye products for baking.

One method for applying this test utilizes the MacMichael viscosimeter. A suspension of rye meal in distilled water at 30°C is heated to 75°C in 2.5 min at a uniform rate by heating the viscosimeter cup and contents in a water bath and using the spindle as a stirrer. The suspension is removed from the water bath and allowed to set for 2.5 min. The viscosity is then measured at 75°C.

The distribution of the viscosity data into the satisfactory and unsatisfactory classifications on the basis of baking tests is shown in Figure 1 for some 500 rye meals. The region where the two curves overlap is small, thus showing a definite correlation between gelatinized meal viscosity and baking quality under the specific test conditions employed.

The utility of the Amylograph for evaluating baking quality of rye meals was next investigated. For this purpose, 13 rye samples of varying baking quality were ground to such a granulation that 95% passed through a No. 16 wire sieve and 50% through a No. 30 wire sieve. A suspension containing 72 g of the meal (dry basis) in 400 ml of  $\text{KH}_2\text{PO}_4$ -NaOH buffer solution of pH 6.2 was placed in the Amylograph bowl, the machine started at 25°C, and the temperature allowed

to increase at the uniform rate of  $1.4^{\circ}\text{C}/\text{min}$  automatically provided by the instrument. The maximum viscosity was read from the curve in Brabender units (B.U.). The diastatic activity of the meals was measured as outlined in Cereal Laboratory Methods (4th ed., 1941). Rye muffin tests were used to evaluate baking quality.

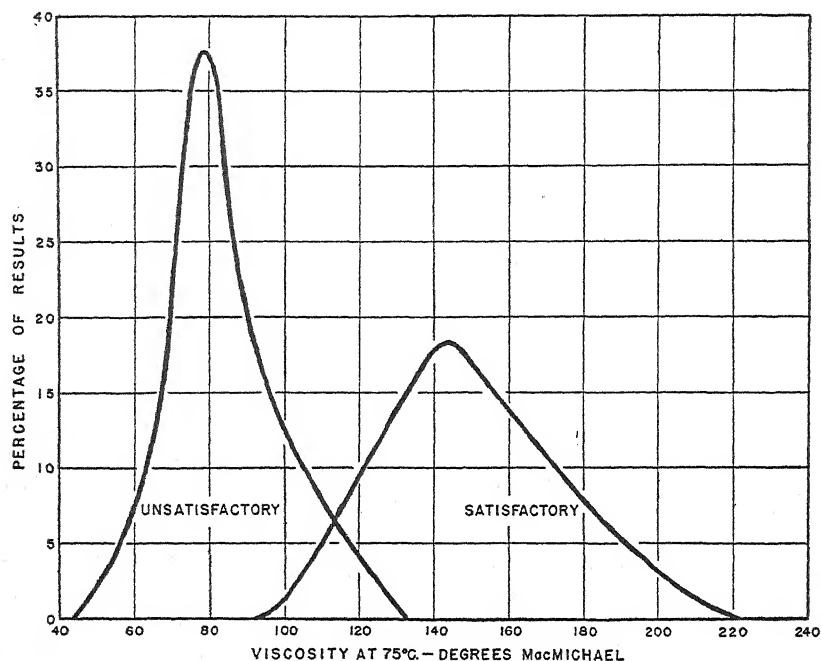


Fig. 1. Division of MacMichael viscosity data for rye meals into satisfactory and unsatisfactory values on basis of baking tests.

The results in Table I show that the higher the maximum viscosity, the better the baking quality, and the lower the diastatic activity. In other words, it is possible by means of the Amylograph to predict the baking quality and thereby furnish a basis for evaluating rye grain.

*Effect of Starting Temperature, pH, Granulation, and Heat Treatment on Maximum Viscosity of Rye Meal Pastes.* In testing rye products in the Amylograph, a period of about 22 min elapses before any increase in viscosity is registered. Different machine starting temperatures were used to ascertain whether this preliminary period could be reduced. The investigations were performed on rye meals using the same conditions as in the previous experiment.

Table II shows that the maximum viscosity is substantially unaltered until the starting temperature is raised above  $40^{\circ}\text{C}$ . Anker and Geddes (1944)<sup>1</sup> obtained a similar result in an analogous investigation

TABLE I  
RELATION BETWEEN BAKING QUALITY, MAXIMUM VISCOSITY,  
AND DIASTATIC ACTIVITY OF RYE MEALS

Rye sample	Baking quality	Maximum viscosity	Diastatic activity mg maltose/10 g
		<i>B.U.</i>	
1	Very poor	150	540
2	Very poor	210	455
3	Poor	223	445
4	Fair	315	395
5	Poor	345	417
6	Fair	435	335
7	Good	442	365
8	Fair	445	382
9	Good	545	323
10	Good	547	352
11	Very good	763	275
12	Very good	1000+	268
13	Very good	1000+	85

with wheat starch, but the effect upon maximum viscosity of further increases in starting temperature was much less marked in their experiments. An increase of the starting temperature from 25°C to 40°C decreases the time required to make a curve on rye meal suspensions from 28 min to 12 min.

TABLE II  
EFFECT OF STARTING TEMPERATURE ON MAXIMUM VISCOSITY OF RYE MEALS

Starting temperature	Rye meal No. 1		Rye meal No. 2	
	Max. viscosity	Time	Max. viscosity	Time
°C	<i>B.U.</i>	<i>min</i>	<i>B.U.</i>	<i>min</i>
25	150	28	545	28
30	155	24		
40	145	10	550	12
50	260	8	1000—	9
60	350	6		
70	375	5		

The effect of pH on maximum viscosity was investigated with suspensions of a corn starch and a medium rye flour brought to various pH values by means of  $\text{KH}_2\text{PO}_4$ -NaOH buffer mixtures. The results are presented in Figure 2. Decreasing the pH of the rye flour suspensions over the range studied markedly decreased the maximum paste viscosity; since there was no effect with corn starch this result must be attributed to increased alpha-amylase activity as the pH was decreased. The maximum viscosity of the rye flour paste at pH 7.0 was almost 250% greater than at pH 5.8; thus it is obviously necessary to control pH in evaluating rye flours by this method.

The effect of granulation on maximum viscosity was investigated by preparing rye meals of two granulations from one lot of rye. One sample of the grain was ground so that 95% passed through a No. 16 wire sieve and approximately 50% passed through a No. 30 wire sieve; the other sample was ground so that 95% passed through a No. 30

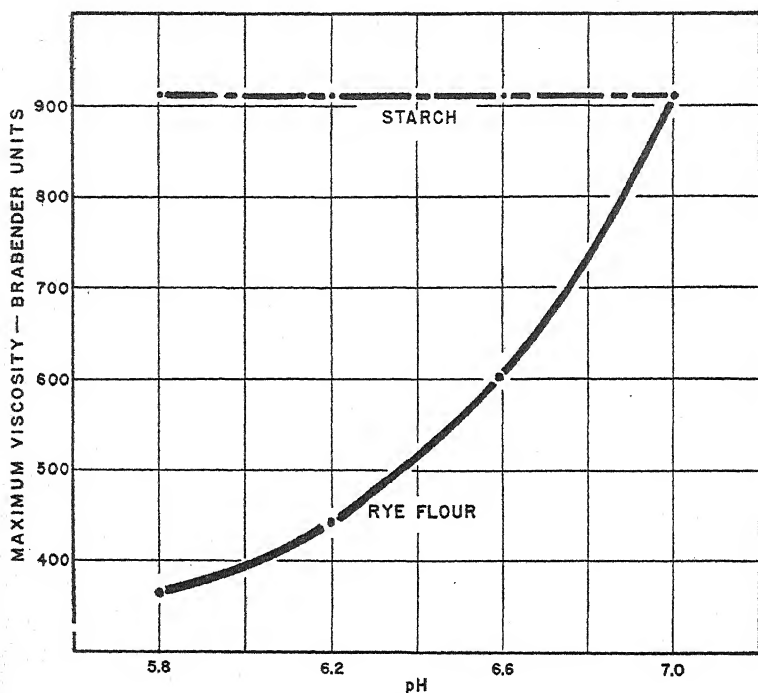


Fig. 2. Effect of pH on the maximum viscosity of a rye flour and a corn starch paste.

wire sieve. Amylographs were made for both meals, employing 72 g in 400 ml  $\text{KH}_2\text{PO}_4\text{-NaOH}$  buffer solution of pH 6.2 and a starting temperature of 25°C. The diastatic activity of each meal was also determined. The results were as follows:

	Coarse meal	Fine meal
Maximum paste viscosity, B.U.	320	535
Maltose value, mg/10 g	323	447

These data show the necessity of controlling granulation in evaluating different samples of rye by this test.

The marked effect of amylase activity on baking quality and maximum paste viscosity of rye meals was further shown by heating a sample of rye meal of poor baking quality in live steam at 120°C for

30 min. The results of diastatic activity, Amylograph, and baking tests for the control and the heated samples were:

	Control	Heat-treated
Maximum viscosity, B.U.	150	1000+
Maltose value, mg/10 g	540	85
Baking quality	Poor	Good

The higher maximum viscosity and better baking quality of the heat-treated sample are associated with a decrease in amylase activity due to heat inactivation of the enzyme.

*Sensitivity and Replicability of the Amylograph.* As a test of the sensitivity of the Amylograph, tests were made with corn starch suspensions in which the weight of starch was varied 2.5 and 5.0% above and below a reference weight of 40 g per 400 ml of buffer solution ( $\text{KH}_2\text{PO}_4$ -NaOH mixture) of pH 6.2. The results in Table III show

TABLE III  
VARIATION OF MAXIMUM VISCOSITY AS RELATED TO  
VARIATION OF WEIGHT OF STARCH

Weight of starch		Maximum viscosity	
Grams	Variation	B.U.	Variation
	%		%
42	+5.0	990	+11.5
41	+2.5	935	+ 5.3
40	0	890	0
39	-2.5	820	- 7.6
38	-5.0	780	-12.2

that, on a percentage basis, the maximum viscosity values vary approximately twice as much as the variation in weight.

As a test of the precision with which the maximum viscosity of rye meal pastes could be determined, Amylograph tests were made in quintuplicate on 11 meals, employing the standard technique previously outlined. The results presented in Table IV show that the precision is quite satisfactory, especially when the magnitude of the viscosity values are taken into consideration.

*Effect of Inorganic Salts on Maximum Paste Viscosity.* As certain electrolytes are known to influence enzyme activity and also to affect starch paste viscosity, Amylograph curves were made with suspensions containing 72 g of a rye meal in 400 ml of 0.1% solutions of various salts at a pH of 6.2 (obtained with  $\text{KH}_2\text{PO}_4$ -NaOH buffer mixture).

The curves for those salts which influenced maximum paste viscosity are reproduced in Figure 3. The cupric, mercuric, cyanide, selenite, borate, and chromate ions increased, and calcium and stannous ions decreased, maximum paste viscosity. Other salts tried but which

TABLE IV  
REPLICABILITY OF MAXIMUM VISCOSITIES ON RYE MEALS

Rye meal	Mean	Minimum	Maximum	Standard error (single deter- mination)	Coefficient of variation
	<i>B.U.</i>	<i>B.U.</i>	<i>B.U.</i>	<i>B.U.</i>	%
1	150	145	155	3.5	2.3
2	223	200	235	14.0	6.3
3	210	200	220	10.0	4.8
4	315	305	330	11.7	3.7
5	345	325	375	20.9	6.0
6	435	425	470	19.7	4.5
7	442	425	470	17.2	3.9
8	445	430	460	11.2	2.5
9	545	515	580	25.5	4.7
10	547	510	580	28.6	5.2
11	763	735	800	27.3	3.6
All samples	402			18.8 <sup>1</sup>	4.7

<sup>1</sup> Computed from the results of a variance analysis of the data.

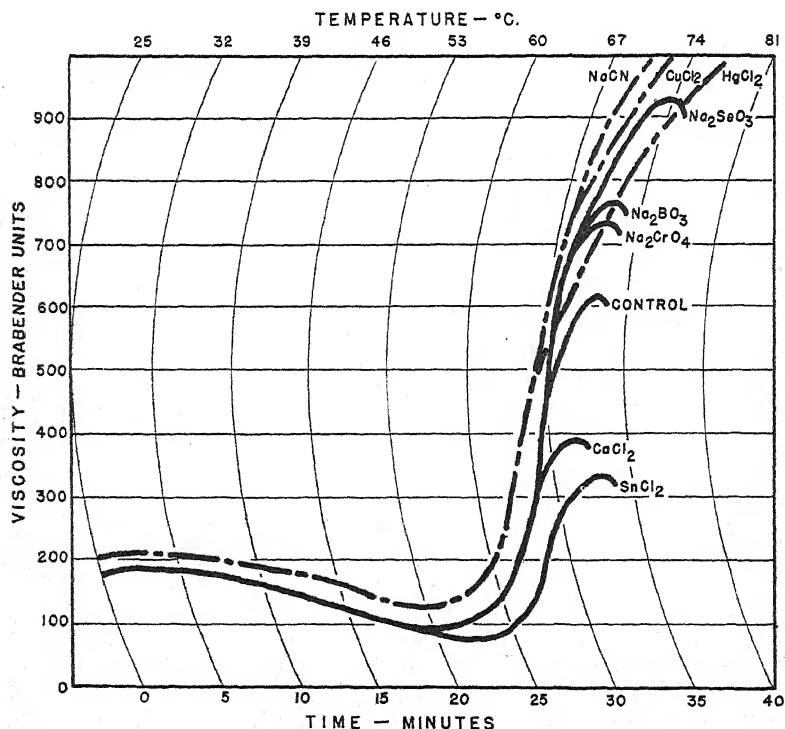


Fig. 3. Amylograph curves for suspensions of rye meal in 0.1% solutions of various salts. Buffer solution was used to obtain a pH of 6.2.

showed little or no influence on paste viscosity in 0.1% concentration were sodium chloride, barium chloride, ammonium chloride, ferric chloride, zinc chloride, potassium iodide, potassium bromate, sodium fluoride, lead nitrate, sodium arsenate, sodium tungstate, sodium citrate, sodium sulfate, and sodium nitrate.

*Effect of Heat Treatment of Soya Meal on Maximum Viscosity.*

Since the heat processing of soya products is said to denature the proteins, thereby influencing their viscosity, experiments were con-

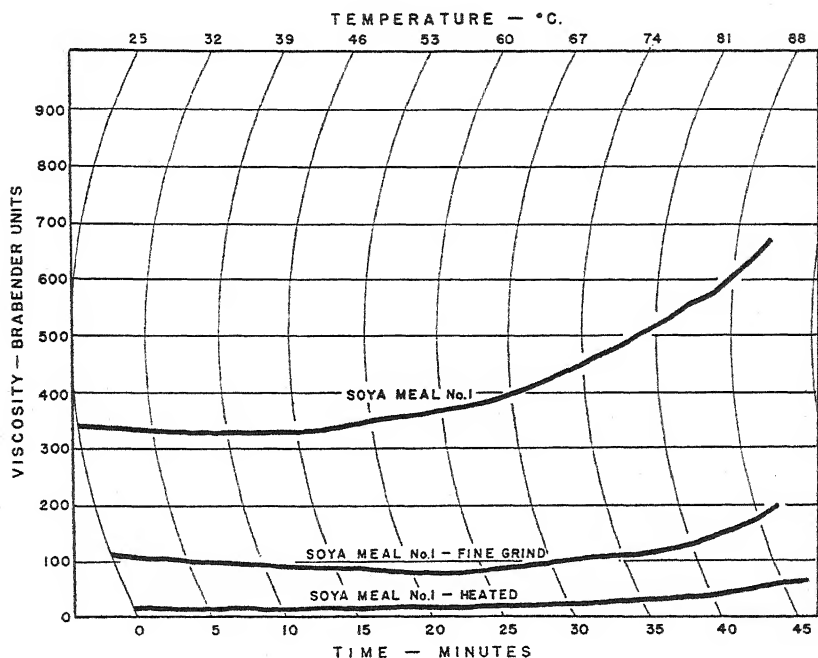


Fig. 4. Showing the effect of granulation and heat processing upon the Amylograph curve for soya meal.

ducted to determine the magnitude of this effect. A soya meal, ground to pass through a No. 18 wire sieve, was divided into three subsamples, one of which served as a control. The second subsample was processed in live steam at 120°C for 15 min, and the third was ground in a ball mill until it passed through a 13XX sieve. Amylograph curves were made by suspending 92 g of the meals in 450 ml of a  $\text{KH}_2\text{PO}_4$ -NaOH buffer solution at pH 6.2. A starting temperature of 25°C was used. Figure 4 shows that both fine grinding and heat processing lower the maximum viscosity. In certain chemically leavened products such as pancakes, it is necessary to employ soya products which give low viscosities.



*Effect of Wheat Type and Amylase Activity on Maximum Paste Viscosity for Wheat Flours.* Amylograph curves were made with a soft wheat flour, southwestern hard wheat flour, and a northwestern wheat flour which gave diastatic activity values of 112, 328, and 395 mg maltose/10 g of flour respectively. The first two flours were also diastated to increase their respective maltose values by 70 units. In making the curves, 45 g of the soft wheat flour, 63 g of the southwestern flour, and 77 g of the northwestern flour were respectively suspended in 400 ml of a  $\text{KH}_2\text{PO}_4$ -NaOH buffer at pH 6.2.

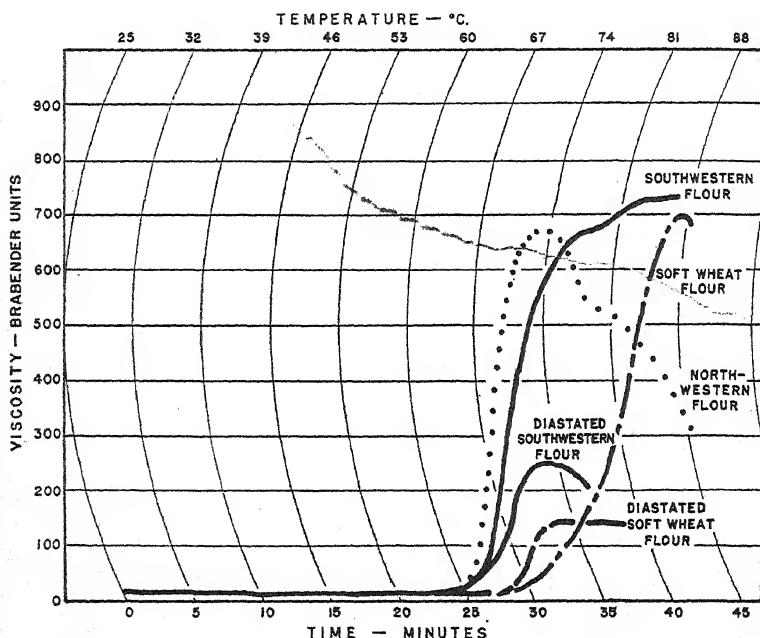


Fig. 5. Amylograph curves for different types of wheat flours. The effect of increasing the diastatic activity of two of the flours by 70 maltose units is shown.

The curves shown in Figure 5 illustrate the marked effect of diastating in decreasing the paste viscosity. They also indicate distinct differences between the amylolytic susceptibility of the soft flour and southwestern hard wheat flour. Anker and Geddes (1944)<sup>1</sup> have carried out Amylograph studies with undiastated and diastated starches prepared from different classes of wheat. They found that the maximum paste viscosity for the undiastated starches differed materially and concluded that maximum paste viscosity cannot be interpreted as a direct index of amylase activity. The limited experiments reported here lend support to this view.

### Summary

Maximum paste viscosity of rye meal suspensions, as measured by the Amylograph, can be employed to classify rye into groups which differ materially in baking value. The granulation of the meal and the pH of the suspension influence the viscosity values and must be controlled. With rye meals, the standard error of a single determination of maximum paste viscosity was 18.8 Brabender units. The time required for the test may be shortened by employing a starting temperature of 40°C rather than the customary temperature of 25°C. Starting temperatures above 40°C give increased paste viscosity values.

In addition to evaluating rye samples, the Amylograph is well adapted for investigations of the effect of inorganic salts on paste viscosity, for determining the effect of granulation and heat processing on the viscosity of soya products, and for viscosity studies on wheat flours.

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## A NOTE ON THE PRESENCE OF FIBER IN THE "AMYLODEXTRIN" FRACTION OF WHEAT FLOUR

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(Received for publication January 3, 1944)

In the course of experiments on starch manufacture, starch suspensions obtained by washing gluten from flour-water doughs were strained through silk 8XX. There remained on the silk a small quantity of a whitish sludge which obviously was not starch. Microscopic examination revealed that it consisted mainly of fragments of thin cell walls with considerable bran powder and some starch embedded in the mass. As this material showed a certain similarity to the "amylodextrin" fraction described by Sandstedt, Jolitz, and Blish (1939),<sup>1</sup> the top layer of a centrifuged starch suspension was examined microscopically. It was found to consist of the same kind of cell wall fragments that made up the sludge, but it contained more starch, and for this reason the bran powder was more dilute. The starch granules embedded in this top layer were of much smaller average size than those found in the bottom layer.

Owing to the removal of the sludge, the volume of the top layer decreased considerably when the starch suspension was strained

<sup>1</sup> Sandstedt, R. M., Jolitz, C. E., and Blish, M. J. Starch in relation to some baking properties of flour. *Cereal Chem.* 16: 780-792, 1939.

through fine bolting silk previous to centrifuging. By repeated straining through bolting silk of increasing fineness, 5XX, 8XX, 10XX, practically all the cell wall fragments could be removed. On centrifuging the strained starch suspension, a top layer of much smaller volume than that of the original one was obtained, but now it consisted almost exclusively of small starch granules. Staining with 0.2 solution of congo red greatly helped to distinguish the cell wall fragments on the slide.

These findings suggested that the unidentified matter referred to by Sandstedt *et al* is cellulose. To verify this assumption, crude-fiber determinations were made on a flour and on the starch and "amylo-dextrin" layer obtained from it. The results, on a 12% moisture basis, were flour 0.34%, starch 0.19%, and amylo-dextrin 0.85%. As the flour yielded 58% starch and 18% amylo-dextrin, 0.11 g of the total fiber is present in the starch, and 0.15 g in the "amylo-dextrin" fraction.

The observations and figures reported in this note show that the "amylo-dextrin" layer cannot be regarded as a distinct chemical fraction. Sandstedt *et al* allowed for this possibility by using the term "amylo-dextrin" only tentatively.

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## SOURCES OF ERROR IN THE DETERMINATION OF THE PROTEIN CONTENT OF BULK WHEAT

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From the time of sampling a car of wheat until the analysis is completed, there are numerous actual or potential sources of error which contribute to lack of agreement between protein determinations made on the same wheat by different laboratories. The present study was designed to afford an estimate of the relative importance of these several sources under normal working conditions. The factors studied were (1) heterogeneity of bulk wheat; (2) sampling error; (3) variation in cleaning procedure; (4) differences in grinding methods; and (5) analytical error.

### Experimental

*Heterogeneity of Wheat.* Because individual wheat kernels from the same sample may differ widely in protein content as in other properties, it is impossible to take two identical subsamples of reason-

able size from a single bulk lot of grain, no matter how careful the blending and sampling may be. In an attempt to estimate the magnitude of this variation, 16 samples of clean wheat were thoroughly blended in a MacLellan mixer and divided into two equal portions by means of a Boerner sampler. One of the subsamples was ground in its entirety, blended, and analyzed in triplicate. The second subsample was divided into three equal portions and each portion was then ground and analyzed. The mean squares for the errors (within samples) of the two procedures were found to be 0.0042 and 0.0103 respectively. If it is assumed that grinding and blending the entire subsample produced a completely homogeneous material, then the value 0.0042 represents analytical variation alone, and the difference between the two mean squares ( $0.0103 - 0.0042$ ), or 0.0061, is a measure of variability in clean, carefully blended wheat. This gives a standard error of 0.078% protein. In other words, if a series of samples were withdrawn from a bulk lot of clean wheat and were then analyzed with perfect accuracy, the results would still be scattered in such fashion that about one third of the values would vary from the mean by more than 0.078% protein.

*Sampling Error.* In addition to the error resulting from variation in wheat, there may be some variation arising from differences in technique when two operators sample the same car or from the fact that bulk lots may not be thoroughly mixed. To estimate the importance of this source of error, 16 cars representing random commercial shipments were sampled by two operators, each using his customary procedure. The duplicate samples were then cleaned and analyzed in triplicate.

As would be expected, the data revealed no systematic difference between operators. However, an appreciable random variation between pairs of samples was found. When other sources of error were accounted for, it was found that the mean square for differences in sampling technique and heterogeneity of wheat amounted to 0.0417. Assuming that the value found for variation in wheat alone also applies in this case, the mean square for sampling amounts to  $0.0417 - 0.0061$ , or 0.0356. The corresponding standard error is then 0.189% protein.

*Cleaning Procedure.* All laboratories do not use the same kind of equipment for cleaning wheat samples prior to analysis, and the amount of small kernel wheat, weed seeds, and the like which is removed may have an appreciable bearing on the final protein content. An experimental determination of the importance of variation in cleaning techniques was made by carefully blending 16 uncleaned wheat samples, dividing them on a Boerner sampler, and cleaning the subsamples, in each of two laboratories, by the procedures customarily

employed. The cleaned samples were then reduced, ground, and analyzed in the same laboratory. After the effect of other sources of variation was removed by appropriate analysis, it was found that the standard error of a single determination for this source of variability was 0.182% protein.

*Grinding Methods.* It was assumed that different laboratories do not employ identical procedures in the grinding of whole wheat samples prior to analysis. The differences in practice may well contribute to variability in results. To measure the magnitude of this source of error, eight clean wheat samples were carefully blended, subdivided by means of the Boerner divider, and sent to each of 10 laboratories to be ground and analyzed. Information was available on the magnitude of analytical error among these laboratories. It was assumed that, after deducting variation between samples, the residual scattering was due to three sources, namely, heterogeneity of wheat, analytical error, and variations in grinding technique. Since the first two factors had been evaluated, the error arising from their operation was removed from the total, leaving a standard error for grinding of 0.165%.

*Analytical Error.* All laboratories will not agree exactly when determining the protein content of a completely homogeneous sample. Data were available for the interlaboratory variation of the 10 laboratories mentioned in the preceding paragraph from collaborative analysis of eight well-blended flour samples. It has been assumed that these samples were entirely uniform and that any variation in results would be due to differences in analytical technique alone. The standard error of a single determination for this source of difference between laboratories was found to be 0.110% protein. This value agrees well with the estimate of interlaboratory error, 0.10%, found by Davis and Wise (1933) and for the intralaboratory variation, also 0.10%, reported by Geddes and Milton (1939).

### Discussion

The findings discussed above are summarized in Table I. Of the five sources of error investigated, it is apparent that differences in sampling, cleaning, and grinding technique are the most important in contributing to variation in wheat protein results. The variation ascribed to heterogeneity of wheat of necessity represents the irreducible minimum of scatter which would be obtained if all other errors were eliminated. Of the four remaining sources of variation, cleaning technique and grinding procedures seem to offer the greatest opportunity for improvement. The equipment can be better standardized

and procedures more carefully controlled. Sampling techniques are fairly well established, and it seems probable that the variation found is due not so much to technique as to lack of uniformity in the bulk wheat.

TABLE I  
INTERLABORATORY ERRORS IN WHEAT PROTEIN DETERMINATIONS

Source of error	Standard error of single determination
	%
Heterogeneity of wheat	0.078
Sampling	0.189
Cleaning	0.159
Grinding	0.165
Analysis	0.110
All sources combined	0.307

The various sources of error are not additive in the form of standard errors. However, they may legitimately be added as squares of these errors. When this is done and the square root of the sum extracted, the over-all standard error is found to be 0.307%. Thus if a car of wheat is sampled by two individuals and the samples are cleaned, ground, and analyzed by a single determination in two laboratories, the results will differ by more than 0.31% about one third of the time. This agreement will not be substantially improved by carrying out duplicate determinations on the ground sample because the analytical error is a relatively small portion of the total. It can be estimated that the standard error will be reduced only to 0.297% protein.

As with all evaluations of this kind, the results listed above have an element of uncertainty inasmuch as the study was carried out with a limited number of samples. The values obtained for error due to differences in grinding technique and to analytical variation are probably more generally reliable than are the other factors since they were calculated from data accumulated by 10 laboratories in contrast to the two laboratories involved in the measurement of sampling and cleaning errors. It is believed, however, that the estimates are reasonably accurate, for the over-all error agrees well with that found in actual practice. Data were available on the analysis of 158 cars of wheat by the two laboratories whose data were used to evaluate sampling and cleaning errors. These laboratories were also included in the studies designed to estimate the other errors. The over-all variation for these 158 pairs of determinations was found to give a standard error of 0.29%, a value in good agreement with the figure of 0.297% calculated from the laboratory studies.

### Summary

Interlaboratory variation in determining the protein content of bulk wheat is caused chiefly by differences between replicate samples. Differences in technique in preparing samples for analysis (cleaning and grinding) are somewhat less important causes, while analytical error is relatively small. Accordingly, little is gained in precision by carrying out replicated analyses of the same subsample of ground wheat. Improvement in results should rather be sought in better sampling and cleaning techniques and in a more uniform grinding procedure.

### Acknowledgments

For their active and generous assistance in carrying out the work reported in this paper we are indebted to Mr. W. R. Young and Mr. John Dwyer of the Minnesota State Grain Inspection Department; and to Messrs. F. A. Collatz, E. V. Hetherington, C. L. Moore, George Swindaman, W. M. Tinkham, E. F. Tibbling, A. A. Towner, B. H. Kepner, A. C. Conner and L. Reimers of General Mills, Inc.

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## THE EFFECT OF TEMPERATURE DIFFERENCES ON SOME MIXOGRAM PROPERTIES OF HARD RED SPRING WHEAT FLOURS<sup>1</sup>

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The effects of temperature variations on the physical properties of dough have been described by a number of workers. Harrel (1927), when determining the stiffness or toughness of doughs with a gravimetric penetrometer, observed that the viscosity decreased with increasing temperature. Skovholt and Bailey (1932) demonstrated an increase of mobility with temperature rise, the variations amounting to 12 to 40 Farinograph units per degree C, depending on the stiffness of the doughs.

Bohn and Bailey (1936) observed the effect of raising the temperature in reducing Farinograph and stress readings. The latter were reduced approximately one third. Halton and Scott Blair (1937)

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<sup>1</sup> Published with the approval of the Director of the Station.

found the viscosity of doughs, as ascertained by the stress extensimeter, to fall about 10% for each degree C rise of temperature. Stamberg and Bailey (1940) pointed out the need for accurate temperature control when measuring dough plasticity with a plastometer, as small temperature variations affected the rate of flow to a greater extent than any probable pressure differences. Moore and Herman (1942) studied the effect of temperature on three Farinograph properties. These were the initial phase, period of resistance, and a factor X calculated from these. As temperature increased, the initial phase and X decreased, while the period of resistance lengthened.

As increasing attention has been given in this laboratory to the estimation of certain properties of mixograms as supplementary criteria of baking quality, it was thought advisable to inquire into the influence of mixing temperature on these properties.

Mixograms were made over a temperature range of 35°C with four hard red spring wheat flours adjusted to two protein levels. In addition, the influence of temperature differences on curve pattern was compared with the effects produced by varying the absorption.

### Materials and Methods

Four long-patent flours experimentally milled from hard red spring wheats of the 1942 crop were employed in the study. These wheats consisted of one sample each of Thatcher, Rival, Vesta, and No. 2822 and were free from visible forms of damage. Comparative analytical data for the four wheats and the resultant flours are shown in Table I.

TABLE I  
COMPARATIVE ANALYTICAL DATA FOR THE FOUR HARD RED SPRING WHEATS AND LONG-PATENT FLOURS

Variety <sup>1</sup>	Test weight	Wheat protein <sup>2</sup>	Flour yield	Flour ash <sup>2</sup>	Flour protein <sup>2</sup>	Absorption	Loaf volume
	lb/bu	%	%	%	%	%	cc
Thatcher	62.3	13.9	71.4	0.41	12.9	62.1	665
Vesta	63.7	13.9	75.6	0.45	12.8	61.8	680
No. 2822	61.7	14.9	74.0	0.44	14.1	62.8	700
Rival	63.2	14.4	70.6	0.50	13.2	62.8	735

<sup>1</sup> All samples graded 1 heavy dark northern spring.

<sup>2</sup> Expressed on 13.5% moisture basis.

Characteristic mixograms of these varieties at the original flour protein content are shown in Figure 1. Wheat No. 2822 is a new unnamed variety with a short dough development and dough stability time, but generally has high protein content and good loaf volume. Vesta, on the other hand, tends to have an exceptionally long dough development period and is relatively resistant to overmixing. Thatcher and



Rival are somewhat similar in their comparative mixing patterns and could be considered approximately average for hard red spring wheat varieties.

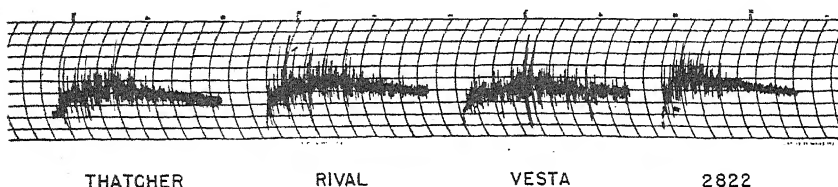


Fig. 1. Comparative mixograms for flours representing the four varieties at the original protein level, using a temperature of 30°C.

The four flours were each diluted to uniform protein levels of 10% and 12% with the same sample of experimentally prepared wheat starch.

In studying the effect of temperature differences on curve pattern, mixograms were made with two formulas: one, a flour-water mix, and the other, flour with the ingredients of the malt-phosphate-bromate formula. Twenty-five g of flour was used throughout. A constant absorption, as determined when baking the samples, was employed for each flour. Mixograms were made at eight temperatures which covered a range of 35°C. It was found convenient to make one determination at the low level of 5°C as a room was available which is held at this temperature. The mixograph, with the cabinet completely opened up, and accessory equipment was placed in the room for some time before the experiment was started, and the work was performed in it. The other temperatures were obtained by a combination of room and mixograph cabinet control. A Bahnson laboratory humidifier was installed in the mixograph cabinet to maintain the relative humidity at approximately 75% throughout the experiment. Temperatures were checked throughout the experiment and care was taken to warm or cool mixograph bowls, flours, and solutions to the required point before mixing.

To determine whether changes in flour absorption would have the same effect on curve pattern as differences in temperature, mixograms were made at 30°C for one flour, employing the baking formula at absorptions of 56, 62, and 68%.

The particular mixogram properties selected for measurement were dough development stage, curve height, and curve width. Dough development stage and curve height have been described by Harris (1943) with methods of estimation. The first depends upon the horizontal distance from the commencement of mixing to the point corresponding to the curve peak or point of optimum development, while

curve height is denoted by the length of the line drawn through the peak point to the edge of the chart paper. The width was estimated from the curve at the point corresponding to maximum height.

### Experimental Results

*Effect of Variations in Temperature on Mixogram Pattern.* The mixograms obtained with the four flours adjusted to a protein level of

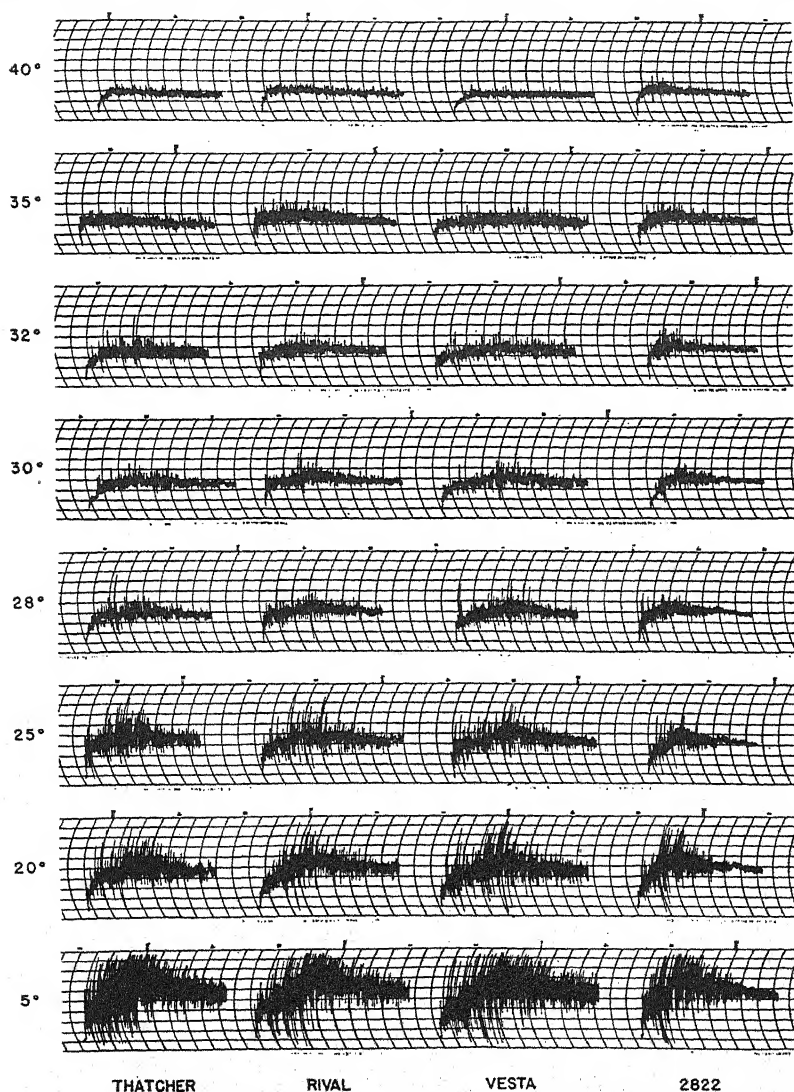


Fig. 2. Effect of mixing temperature on the mixogram patterns for flours representing four hard spring wheat varieties. The flours were diluted with wheat starch to a constant protein level of 12.0%. Mixograms were made with flour and water only.

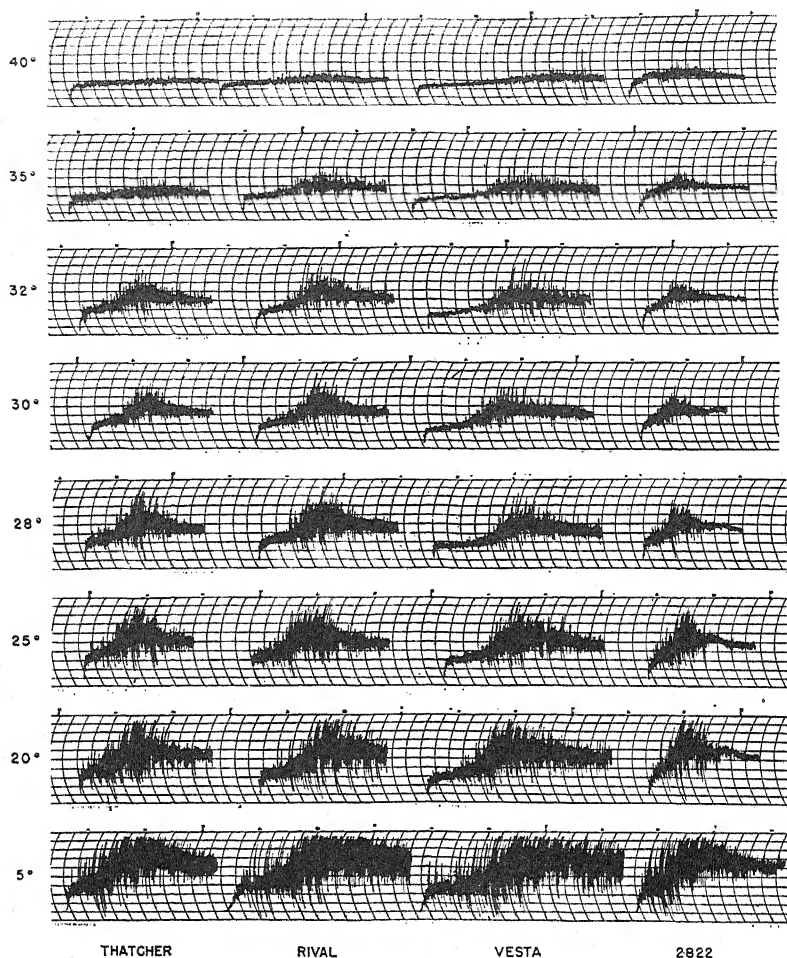


Fig. 3. Effect of mixing temperature on the mixogram patterns for flours representing four hard red spring wheat varieties. The flours were diluted with wheat starch to a constant protein level of 12.0%. Mixograms were made with flour and the ingredients of the malt-phosphate-bromate baking formula.

12% are reproduced in Figures 2 and 3. The mean values for dough development stage, curve height, and curve width for each temperature and each flour (variety) are shown in Table II, together with a variance analysis of the data.

For all three curve properties except dough development stage for the baking-formula doughs, the values markedly decrease with an increase in the temperature at which the mixograms were made. The decrease is fairly consistent for curve height and width for both the formulas and is substantially greater for curve height. With the bak-

ing-formula doughs, the initial decrease in dough development with an increase in temperature is followed by a sharp increase at temperatures above 30°C; this is due to the exceptionally long gradual rise in curve height to the peak. For all curve characteristics, a difference of 5°C

TABLE II  
MEAN VALUES OF SOME MIXOGRAM PROPERTIES AS INFLUENCED BY  
TEMPERATURE DIFFERENCES AND WHEAT VARIETY  
FLOURS ADJUSTED TO A UNIFORM PROTEIN CONTENT OF 12.0%

Temperature	Dough development stage		Curve height		Curve width	
	Flour-water mixes	Baking <sup>1</sup> formula mixes	Flour-water mixes	Baking <sup>1</sup> formula mixes	Flour-water mixes	Baking <sup>1</sup> formula mixes
°C	cm	cm	cm	cm	cm	cm
40	2.6	13.0	5.3	5.6	0.7	0.9
35	4.9	11.1	5.9	6.5	1.2	1.3
32	5.5	8.9	6.3	7.4	1.2	1.6
30	6.0	8.3	6.7	7.7	1.0	1.7
28	5.5	8.5	6.8	8.0	1.4	1.8
25	5.6	7.7	7.6	8.6	1.8	2.2
20	5.9	8.7	8.2	9.2	2.4	2.0
5	7.2	11.7	10.2	10.6	3.3	2.4

## WHEAT VARIETY MEANS

Vesta	6.9	13.3	7.0	7.7	1.7	1.8
Rival	5.4	10.4	7.2	8.2	1.6	1.9
Thatcher	5.5	9.5	7.1	7.9	1.7	1.7
No. 2822	3.7	5.8	7.2	7.9	1.5	1.6

## ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variances <sup>2</sup>		
		Dough development stage	Curve height	Curve width
Temperatures	7	6.09**	18.83**	3.29**
Varieties	3	77.51**	0.29**	0.15
Mixes	1	301.46**	10.16**	0.17
Interaction:				
Varieties × mixes	3	13.03**	0.05	0.11
Temperatures × varieties	21	0.91	0.09*	0.12*
Temperatures × mixes	7	15.67**	0.24**	0.54**
Temperatures × varieties × mixes	21	1.57	0.04	0.05
Total	63			

<sup>1</sup> The baking formula consisted of the following ingredients in the specified percentages: high diastatic malt 0.3%; ammonium di-hydrogen phosphate 0.1%; potassium bromate 0.001%; sucrose 5.0%; sodium chloride 1.0%; yeast 3.0%.

<sup>2</sup> The interaction, temperatures × varieties × mixes, was used as error.

\* Denotes significance exceeding 5% point.

\*\* Denotes significance exceeding 1% point.

causes distinct changes and even a variation of  $\pm 2^{\circ}\text{C}$  from  $30^{\circ}\text{C}$  produces noticeable differences.

The long dough development period of Vesta and the extremely short development time of No. 2822 are in agreement with the previous observations of Harris, Sibbitt, and Elledge (1944). Varietal differ-

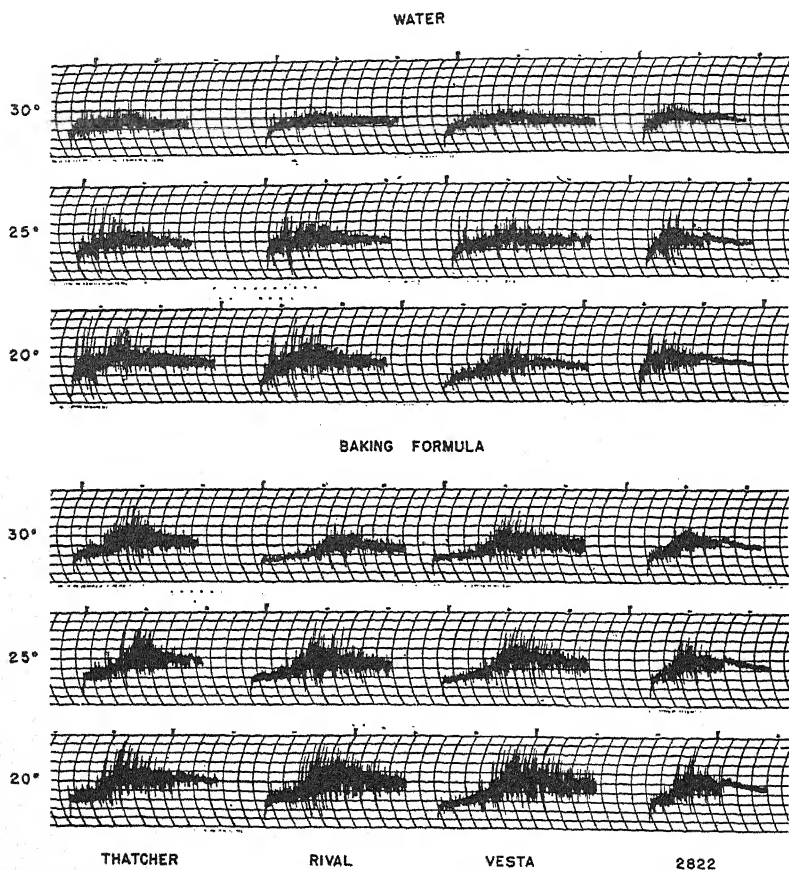


Fig. 4. Effect of mixing temperature on the mixogram patterns for flours representing four hard red spring wheat varieties. The flours were diluted with wheat starch to a protein level of 10.0%.

ences are largely obscured when the curves are made at temperatures which deviate markedly from the normal. Thus at  $40^{\circ}\text{C}$  the mobility of the dough is so great that very little resistance is offered to mixing and the curve is low and extremely narrow; whereas at  $5^{\circ}\text{C}$  the curve is high and very wide. These changes render it difficult to detect differences due to variety. It is apparent that varietal differences are more pronounced when the flours are mixed with the ingredients of the baking formula than when water alone is used. The variance analyses

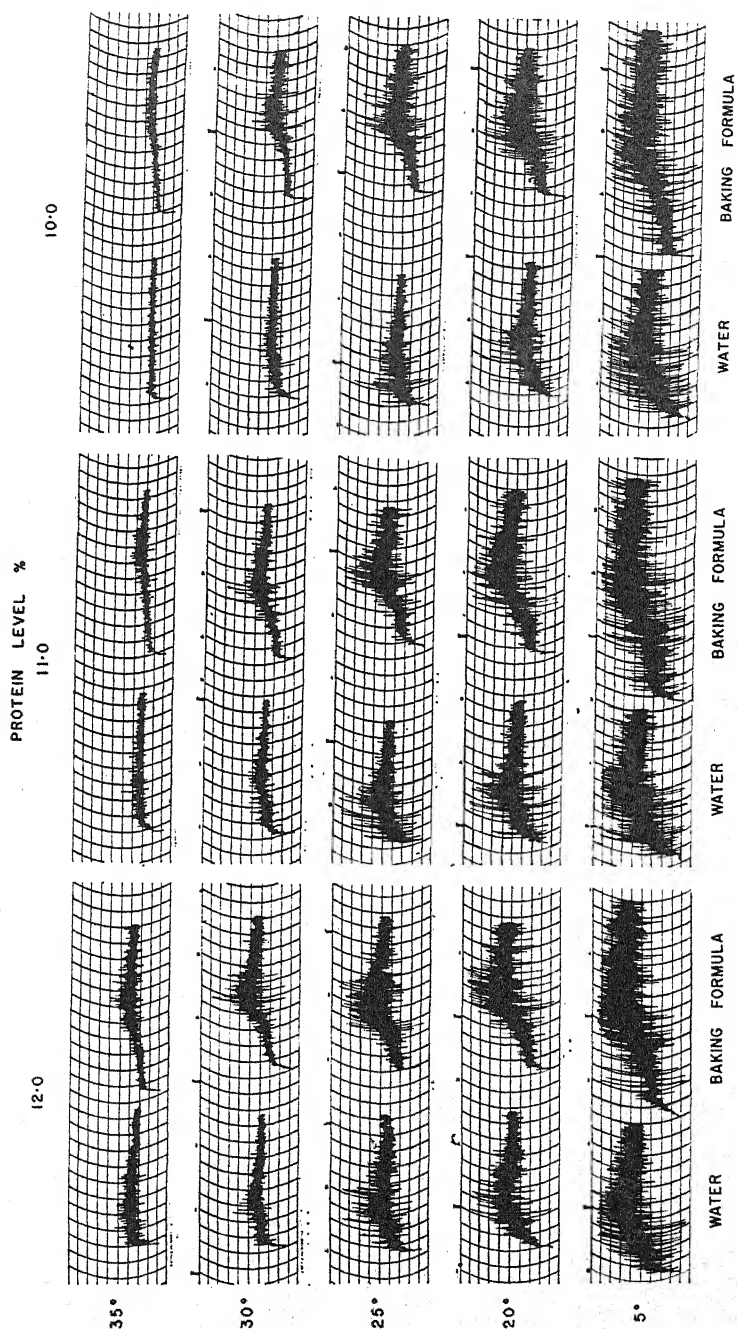


Fig. 5. Effect of flour protein content on microgram patterns. A hard red spring wheat flour was diluted with wheat starch to produce the three protein levels.

show that the effect of temperature on curve height and width is not uniform for the different varieties. The dough development stage, however, varies with temperature in essentially the same manner for all the varieties. Moreover, the precise effect of temperature differs, depending upon whether the doughs are mixed with water or with the ingredients of the baking formula.

Mixographs for the four flours adjusted to the comparatively low protein level of 10% are shown in Figure 4 for temperatures of 20°, 25°, and 30°C. The flour-water curves made at 30°C are quite useless for differentiating between the varieties; for this purpose, those obtained at 20°C are probably the most valuable. With the baking-formula doughs, the authors prefer the mixograms secured at 25°C for varietal differentiation.

In addition, as shown in Figure 5, curves were made with both formulas, at temperatures of 5, 20, 25, 30, and 35°C, employing a hard red spring wheat flour adjusted to protein levels of 10, 11, and 12%. Mean values for the mixogram characteristics for each temperature and protein level are recorded in Table III, together with a variance analysis of the data. The principal additional point of interest in these studies is that the protein level had less effect on curve properties than the variations in temperature which were used. Curve height was the only mixogram characteristic significantly influenced by protein content. As protein content was decreased, the curve height became less. This is also evident from a comparison of the curves given in Figure 4 with those of Figures 2 and 3. Swanson (1941) found that lowering the flour protein reduced curve height but did not affect the varietal pattern of hard red winter wheat flours.

These data show the very marked influence of mixing temperature upon the mixogram pattern and emphasize the importance of close temperature control in mixogram studies.

*The Comparative Effects of Variations in Temperature and Absorption on Mixogram Properties.* Curves made at 30°C with a hard red spring wheat flour and the ingredients of the baking formula employing three absorptions are shown in Figure 6. The normal baking absorption for this flour was 62%. The dough mixed at 68% absorption was very slack, while that mixed at 56% absorption was decidedly stiff. While an increase in absorption, like an increase in mixing temperature, decreased curve height and, to a lesser extent, curve width, the dough development stage was lengthened. The influence of a temperature change on curve pattern is therefore not the same as the effects produced by varying the flour absorption.

TABLE III  
MEAN VALUES OF SOME MIXOGRAM PROPERTIES AS INFLUENCED BY  
TEMPERATURE DIFFERENCES AND PROTEIN LEVEL

Temperature	Dough development stage		Curve height		Curve width	
	Flour-water mixes	Baking <sup>1</sup> formula mixes	Flour-water mixes	Baking <sup>1</sup> formula mixes	Flour-water mixes	Baking <sup>1</sup> formula mixes
°C	cm	cm	cm	cm	cm	cm
35	6.3	11.8	5.8	6.2	0.9	1.2
30	6.5	9.5	6.5	7.3	1.1	1.5
25	5.6	8.2	7.6	8.3	1.8	2.2
20	6.1	9.2	8.0	8.9	2.1	2.4
5	8.1	13.4	9.7	10.1	3.0	2.7

## EFFECT OF PROTEIN LEVEL

Protein level, %						
12	6.0	10.2	7.8	8.7	1.8	2.0
11	6.5	10.5	7.7	8.1	1.8	2.1
10	7.2	10.5	7.0	7.6	1.7	2.0

## ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variances <sup>2</sup>		
		Dough development stage	Curve height	Curve width
Temperatures	4	13.22**	13.64**	3.02**
Protein levels	2	1.42	2.57**	0.03
Mixes	1	113.29**	3.14**	0.36
Interaction:				
Temperatures × mixes	4	2.67*	0.09	0.11
Protein levels × temperatures	8	1.45	0.06	0.15
Protein levels × mixes	2	0.63	0.15*	0.02
Protein levels × temperatures × mixes	8	0.69	0.04	0.13
Total	29			

<sup>1</sup> See Table II footnote 1 for description of baking formula.

<sup>2</sup> The interaction, temperature × varieties × mixes, was used as error.

\* Denotes significance exceeding 5% point.

\*\* Denotes significance exceeding 1% point.

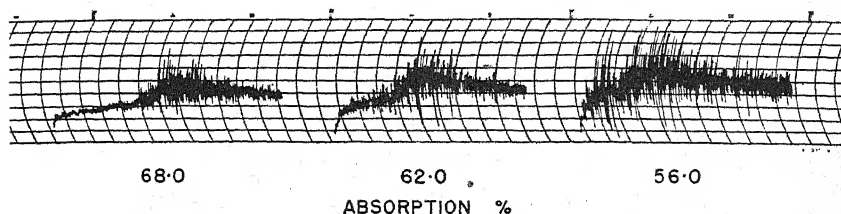


Fig. 6. Effect of absorption on curve pattern at a protein level of 12.0%. Mixograms were made at 30°C with the ingredients of the malt-phosphate-bromate baking formula.



### Discussion

The marked changes in curve pattern as the temperature is increased may be attributed to (1) a decrease in the viscosity of the interstitial water (free water); (2) a decrease in the amount of adsorbed (bound) water, and (3) a decrease in the intensity, or in the relative number of the forces of interaction functioning between the nonwater dough components, particularly the proteins. The relative importance of these factors cannot be separately evaluated. The fact that there is a differential reaction of the varieties to the effect of temperature on curve pattern indicates that the relative importance of the factors just enumerated varies with different flours.

### Summary

The temperature at which mixogram patterns of hard red spring wheat flours are made has an exceedingly important effect upon curve properties. Curve height and width were decreased by increasing the temperature. Dough development period was also markedly reduced by increasing the temperature except in the case of curves made with baking formula doughs at temperatures above 30°C. This appeared to be due chiefly to the low form of the mixograms. Varietal differences were more pronounced when the flours were mixed with the ingredients of the baking formula than when water alone was used.

The precise effect of temperature on curve height and width was not uniform for flours representing different varieties. Dough development stage, however, varied with temperature in essentially the same manner for all varieties. The precise effect of temperature variations on mixograms differed for flour-water and baking formula doughs.

Differences in protein content were less effective in changing mixogram properties than temperature variations. Curves resembling those of normal protein hard red spring wheat flours may be obtained from low protein flours by making the recordings at a lower mixing temperature.

Variations in curve pattern obtained by changing the flour absorption differed somewhat from those caused by a change in temperature. While an increase in absorption decreased curve height, and to a lesser extent curve width, the dough development stage was increased.

### Acknowledgment

The authors wish to acknowledge the valuable assistance of Muriel Elledge in performing the statistical analyses.

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## THE MEASUREMENT OF OVEN SPRING AS AN AID IN CONTROLLING FLOUR QUALITY

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The measuring of oven spring by a device similar to that described by Whitcomb (1938)<sup>1</sup> has proved helpful when used in conjunction with the baking test during the routine testing of bread flour. Since oven spring is indicative of gas production and gas retention in a dough, it is logical that it should serve some purpose in controlling flour quality. The present report shows how this test has been used.

The device used by Whitcomb (1938)<sup>1</sup> for measuring oven spring was refined by making it possible to read the scale to the nearest millimeter. The device is shown in Figure 1. Among the flour samples tested with the device were short patents, stuffed straights, and first clear flours milled from Kansas wheat and patents and first clears milled from spring wheat. All baking tests were made using the Bread Baking Test for Wheat Flours as outlined in *Cereal Laboratory Methods* (4th ed., 1941). All doughs were proofed for the con-

<sup>1</sup> Whitcomb, W. O., 1938. Oven spring of dough as correlated with certain properties of bread. *Cereal Chem.* 15: 206-216.

stant time of 55 min. The measuring of the oven spring was done as recommended by Whitcomb; however, readings were made to the nearest millimeter.

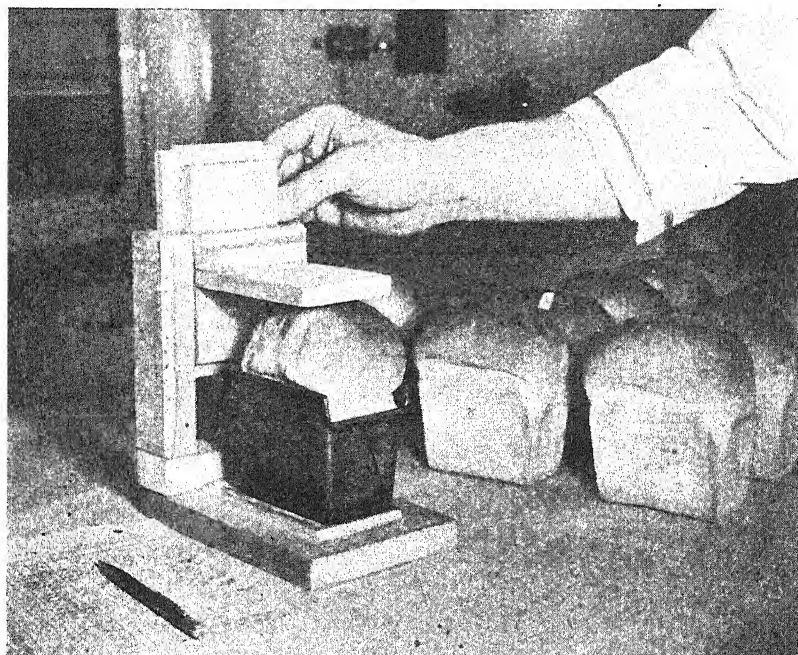


Fig. 1. Device for the measurement of oven spring.

The effect of increasing increments of malted wheat flour on a sample of wheat flour is shown in Table I. As malted wheat flour was added to the control flour, the height of the dough at the end of the proofing period increased. Also, the gassing power, as measured by

TABLE I  
EFFECT OF INCREMENTS OF MALTED WHEAT FLOUR ON PROOFING  
HEIGHT AND GASSING POWER OF DOUGHS

Sample	Height of dough <sup>1</sup>	Gassing power <sup>2</sup>
	<i>mm</i>	<i>mm</i>
Control <sup>3</sup>	92	324
Control plus 0.2% malted wheat flour	100	413
Control plus 0.3% malted wheat flour	101	469
Control plus 0.4% malted wheat flour	103	502
Control plus 0.5% malted wheat flour	103	509

<sup>1</sup> Measured at end of proofing period.

<sup>2</sup> Gas pressure in mm Hg, as measured in the Sandstedt-Blish Pressuremeter during 5 hr fermentation at 30°C.

<sup>3</sup> The control was a non-diastated short patent flour milled from Kansas Wheat. The flour contained 12.0% protein and 0.40% ash, both expressed on a 13.5% moisture basis.

the Sandstedt-Blish Pressuremeter (Cereal Laboratory Methods, 4th ed., 1941), increased as the malted wheat flour was increased. This indicates that the height of the dough at the end of the proofing period depends to a great extent on the gassing power of the flour. Thus, the measurement of the height at the end of the proofing period with the oven spring device gives an indication of the gassing power of the flour. This measurement is not as sensitive as that given by the Sandstedt-Blish Pressuremeter; however, a severe deficiency or excess of gassing power is indicated by the height of the dough at the end of the proofing period.

In Table II, examples are given of two pairs of doughs, each pair having the same height at the end of the proofing period but differing

TABLE II  
RELATION OF OVEN SPRING TO GAS RETENTION PROPERTIES OF DOUGH<sup>1</sup>

No.	Height after proofing	Height after baking	Oven spring	Loaf volume
	<i>mm</i>	<i>mm</i>	<i>mm</i>	<i>cc</i>
1	107	131	24	650
2	107	136	29	695
3	108	140	32	695
4	108	146	38	755

<sup>1</sup> All flours were short patents milled to the same specifications by different mills.

in their oven spring values. In each pair, the same height before going into the oven indicates to a great extent equal gassing power, but the dough having the greater oven spring must have a gluten better able to retain the gas during baking. In this way, the oven spring device is useful in indicating the relative gas retention properties of flour samples milled to the same specifications.

The data obtained from the device during routine test baking on the same type of flour serve at least two purposes:

First, the height of the dough at the end of the proofing period and just before going into the oven is usually indicative of the gassing power of the sample. Although the device has not been found sensitive enough to detect small differences in diastatic activity between samples, it is of service in pointing out flour samples having abnormally high or low gassing power. It should be realized, however, that measurements of height of dough are dependent on the elasticity of the gluten as well as the gassing power. Thus, an estimate of gassing power given by the oven spring device is not a simple measurement of gas evolution.

Second, the measurement of oven spring, which is the difference between the height of the dough before entering the oven and the height of the baked loaf, is of some value in judging the gas retention

properties of flour. As shown in Table II, the evaluation of gas retention properties by the oven-spring device is relative. When samples of flour, milled to the same specifications, are of about the same height before going into the oven, their oven spring values may be compared. With the aid of the oven-spring measuring device, some information in relation to the gas-producing and gas-retaining properties of flour may be obtained during the course of routine test baking.

The additional operations in using the device are few, and abnormal flour samples are quickly noted.

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## REPORT OF THE 1943-44 METHODS OF ANALYSIS SUBCOMMITTEE ON THIAMINE ASSAY <sup>1</sup>

JOHN S. ANDREWS, Chairman

General Mills, Inc., Research Department, Minneapolis, Minnesota

(Read at the annual meeting, May 1944)

The recently completed collaborative study of thiamine assay methods has differed somewhat from those carried out previously. In the past, collaborators have assayed samples by specific procedures and the results have been examined to determine how well the different laboratories agreed. While in many instances the agreement has been quite good, there have been a number of discrepancies which could not be accounted for. This has suggested the desirability of collaboratively studying the separate steps of the assay methods. How critical are these steps, and which might be expected to give rise to errors?

A canvass of 42 laboratories was made to obtain ideas about answering this question. The result was a long list of constructive suggestions covering nearly every phase of the thiochrome procedure. A few of the items were chosen for study. One relates to the efficiency of zeolite for removing interfering substances. Others consider factors relating to the conversion of thiamine to thiochrome; the effect of the amount of ferricyanide, the order of adding ferricyanide and alkali, the period of shaking, and the stability of the extracted thiochrome.

### The Collaborative Committee's Methods

A complete description of the recommended procedures, together with a detailed discussion of the reasons for their selection, is best

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<sup>1</sup> Paper No. 59, Journal Series, General Mills, Inc., Research Department.

afforded in the two communications addressed to the collaborators. For this reason they are reproduced below.

TO THE MEMBERS OF THE A.A.C.C. COLLABORATIVE COMMITTEE  
ON THE THIOCHROME METHOD

Among the numerous questions raised by the collaborators about the thiochrome procedure are several relating to the quantitative aspects of the zeolite treatment of cereal extracts and the validity of the method employed for determining the value of the standard thiamine solutions used in calculating the vitamin content of the assay samples. Since considerable light can probably be thrown on both these problems by a series of recovery experiments, the following is being submitted for collaborative study. Two ampules of a standard thiamine solution containing 0.5 mg per ml (kindly supplied by Dr. Arnold of Winthrop Chemical Company) and samples of enriched flour and bread are being furnished for this work.

### Experimental

One-gram samples of the enriched flour are weighed into each of six 250 ml flasks and suspended in 50 ml of 2% acetic acid containing, respectively, 0, 1, 2, 3, 4, and 5  $\mu$ g of thiamine. To prepare these solutions, dilute 1 ml of the submitted standard to 500 ml with 2% acetic acid and in turn dilute 50 ml of this solution to 250 ml with the same solvent. Dilute 5, 10, 15, 20, and 25-ml aliquots of this second solution to 50 ml with 2% acetic acid and use for the extractions listed above.

Extract by heating on a steam bath for 15 min, taking precautions to prevent evaporation. Cool, add 5 ml of 1.5*N* sodium hydroxide and 5 ml of freshly prepared 6% takadiastase solution. Stopper the flasks and incubate at 37°C for 3 hr. Filter and pass 20 ml aliquots of the filtrates through zeolite in the manner you regularly employ. After washing and drying the zeolite, remove the thiamine with two 10-ml portions of hot 25% KCl in 2% acetic acid, collecting the first 15 ml. Mix thoroughly to insure homogeneity of this solution.

Transfer 5-ml aliquots to glass-stoppered cylinders or reaction vessels and add rapidly one drop (approximately 0.05 ml) of 0.5% potassium ferricyanide solution, 3 ml of 15% sodium hydroxide, and 14 ml of isobutanol, quickly mixing after each addition. Shake vigorously for about 1 min, centrifuge, and discard the aqueous layer. Dry the isobutanol layer with sodium sulfate, transfer the *clear* solution to the curvette, and measure the fluorescence in the regular manner. Due to the rather wide range of thiamine represented by these extracts, the fluorescence of the extract containing no added thiamine should not be too high. A reading of below 30 galvanometer units should keep the fluorescence values of the other extracts on the galvanometer scale. After measuring the fluorescence values of the isobutanol solutions, determine the "blanks" in the usual manner. It will not be necessary to "run blanks" on all, since they should be identical. Two are sufficient unless discrepancies are noted.

Repeat the same experiment, using the sample of bread.

### Notes

It will be greatly appreciated if each collaborator will follow a standard form in reporting his results. This will greatly facilitate subsequent analyses of the data. The attached sample sheet will illustrate the form desired. On it is recorded a typical set of data.

Under the column marked "Test," A and B represent duplicate experiments. Where time permits it is hoped that each collaborator will repeat the assays, preferably on different days, recording the results separately as indicated.

The "Fl.—orig." refers to the fluorescence values of the thiochrome solutions, and column "Fl.—Bl.," the "blank" values. Column III is simply the differences between the corresponding Fl.—orig. and Fl.—Bl. values, and column IV the differences between successive values in column III. These values (IV) represent the actual differences in fluorescence due to added 1- $\mu$ g increments of thiamine, and the averages shown at the bottom of the column are the mean values used to calculate the assays in the manner shown below. Column V shows the amount of fluorescence due to the thiamine in the sample alone after deducting that due to the added vitamin. The first value, 31, is of course that determined directly (column III). The next value, 31.8, is obtained by deducting 7.2, the average increase due to 1  $\mu$ g

## A.A.C.C. COLLABORATIVE STUDY RECOVERY EXPERIMENT—ENRICHED FLOUR

Sample	Test	Fl.— orig.	Fl.— Bl.	III	IV	V
1-g sample	A	37	6	31		31
	B					
Plus 1 $\mu\text{g}$ B <sub>1</sub>	A	45	6	39	8	31.8
	B					
Plus 2 $\mu\text{g}$ B <sub>1</sub>	A	52	6	46	7	31.6
	B					
Plus 3 $\mu\text{g}$ B <sub>1</sub>	A	59	6	53	7	31.4
	B					
Plus 4 $\mu\text{g}$ B <sub>1</sub>	A	65	6	59	6	30.2
	B					
Plus 5 $\mu\text{g}$ B <sub>1</sub>	A	73	6	67	8	31
	B					
<i>Averages</i>	A				7.2	31.2
	B					
<i>Final assay value</i>	A					4.33 $\mu\text{g/g}$ or
	B					1.97 mg/lb

of added B<sub>1</sub>, from the corresponding value in column III. The next value, 31.6, is found by deducting  $7.2 \times 2$  (2  $\mu\text{g}$  of added B<sub>1</sub>) from 46 in column III, etc.

The thiamine content of the sample is readily calculated from the averages in columns IV and V by dividing the latter by the former. Thus,  $31.2 \div 7.2 = 4.33$   $\mu\text{g/g}$ , or 1.97 mg/lb.

It should be pointed out that this study is proposed not as a method for assay but as an experiment to evaluate steps in the present assay procedure. No one would want to run so many levels of vitamin in a single analysis. In the present instance, the five levels of added vitamin are suggested for two reasons: (1) to increase the accuracy of the results and, (2) to study the effectiveness of the method over a fairly wide range of vitamin concentrations. The work can be simplified to some extent by decreasing the number of levels, and where the collaborator feels that this would expedite his work, it is suggested that the 2- and 4- $\mu\text{g}$  levels of added B<sub>1</sub> be omitted. This, with the omission of duplicates, should enable all the collaborators to make some contribution. It is hoped, however, that the full program can be carried out and thereby insure the maximum value from the study.

In comparing the results from the flour and bread samples, it will be of considerable interest to note the relationship between the column IV averages of these two samples. If they are essentially identical, it can be assumed that thiamine is recorded similarly in both types of products. If they differ materially, it will be necessary to re-examine present procedures, at least as they pertain to the use of the assay standard. Your data will help materially to settle this question.

Anticipating that there may be some significant difference between the column IV values for flour and bread, one other short study is proposed. This merely comprises omission of the sample and carrying out the assays on the several levels of pure thiamine alone. How do the column IV values thus obtained compare with those for bread and flour? If you can include such an experiment in your study, it may add much to the significance of the results.

One more request. Just to bring your own methods into the picture, please include in your report an analysis of the two samples, using your own standard thiamine and your present technique.

Compare your standard with the one prepared from the solution submitted for this collaborative study.

In order to have time to assemble and analyze all the collaborators' data and prepare a report for the forthcoming A.A.C.C. convention, your results should be sent in as soon as possible. It will be greatly appreciated if this is done before March 15. Data received after April 1 will be too late for formal inclusion in the convention report.

JOHN S. ANDREWS, Chairman  
Vitamin Assay Committee

TO THE MEMBERS OF THE A.A.C.C. COLLABORATIVE COMMITTEE  
ON THE THIOCHROME METHOD

The replies received from the collaborators in response to the writer's request for comments on phases of the thiochrome method believed to need further study indicated a widespread uncertainty about the present oxidation procedures. Numerous questions have been raised about such factors as (a) optimum strength of the ferricyanide solution, (b) preferred order of adding alkali and ferricyanide, (c) optimum oxidation time and conditions, (d) stability of the thiochrome solution, etc. Because of these queries, one phase of the collaborative study has been designed to examine the above factors.

Two ampules of a standard thiamine solution containing 0.5 mg/ml (kindly supplied by Dr. Arnold of Winthrop Chemical Co.), together with a sample each of enriched flour and enriched bread, are being submitted for this collaborative work.

### Experimental

In order to supply sufficient material for the oxidation studies, four 2-g samples of enriched flour (also enriched bread) are extracted with 50-ml portions of 2% acetic acid and the samples heated in a steam bath for 15 min, taking care to avoid evaporation losses. Five ml of 1.5*N* sodium hydroxide and 5 ml of freshly prepared 6% takadiastase solution are added to the cooled flask and the samples incubated for 3 hr at 37°C. After filtration, the four filtrates are combined and 20-ml aliquots are passed through 8 zeolite adsorption columns. The thiamine is removed from the zeolite with 25% potassium chloride in 2% acetic acid, 15 ml of solution being collected from each tube. These are then combined to give approximately 120 ml of solution for the oxidation studies.

*Effect of Amount of Ferricyanide.* Prepare a 4% solution of ferricyanide and dilute portions of this solution to give concentrations of 2%, 1%, and 0.5%, respectively. Place 5-ml aliquots of the combined zeolite-treated extracts in *glass-stoppered* cylinders or reaction vessels and oxidize, using one drop (approximately 0.05 ml) of these four different concentrations of ferricyanide solutions, adding the ferricyanide before the 3 ml of 15% sodium hydroxide. Add 14 ml of isobutanol in the regular manner, shake for one min, separate the two layers, dry the isobutanol layer with sodium sulfate, and determine the fluorescence. Report the fluorescence values for each of these oxidation experiments together with a blank value for the extract (both flour and bread samples).

*Effect of Order of Adding Reagents.* Following the above procedure, compare the fluorescence values obtained when the lowest (0.5%) and highest (4.0%) concentrations of ferricyanide are added *after* the addition of the sodium hydroxide. Also compare these results with those obtained when both ferricyanide and sodium hydroxide are mixed together before adding to the extracts. The solution for this latter experiment can be conveniently prepared by adding 5 drops of the ferricyanide solution to 15 ml of the sodium hydroxide and employing 3-ml aliquots of this mixture for the oxidation.

*Effect of Time and Shaking.* Using one drop of the 0.5% and the 4.0% ferricyanide solutions and adding the ferricyanide before the alkali, carry out one set of oxidations by merely inverting twice the oxidation mixture of extract, ferricyanide, alkali, and isobutanol. Allow to stand one min before separating the layers and determining fluorescence. Compare the results with those obtained above where one-min shaking was employed. Again using the lowest and highest concentrations of ferricyanide and the procedure in which ferricyanide is added before the alkali, compare the fluorescence values obtained when shaking is carried out  $\frac{1}{2}$  min, 2 min, and 4 min.

*Stability of the Thiochrome Solution.* Using your regular procedure for oxidizing thiamine solutions, oxidize three 5-ml aliquots of the flour and bread extracts, preparing the isobutanol solutions of thiochrome ready for fluorescence measurements. Read the fluorescence of the first at once, and allow the other two to stand on the desk for 30 and 60 min, respectively, before determining the fluorescence values.

### Notes

Since the above experiments are designed to compare different types of oxidation treatments, your report needs only to record the fluorescence values of the isobutanol extracts. Don't attempt to calculate back to thiamine content of the sample for this work. In order to correlate the different collaborators' data, it will be desirable



to record the fluorescence of a standard thiamine solution. For this purpose, please use the submitted thiamine standard, diluting 1 ml (0.5 mg thiamine) to 100 ml with 2% acetic acid, and in turn diluting 10 ml of the resulting solution to 250 ml with 25% potassium chloride in 2% acetic acid. Five-ml aliquots of this potassium chloride solution should be oxidized directly and the fluorescence values of the thiochrome solution and the "blanks" reported.

Please include in your report thiamine values for the samples of enriched flour and enriched bread, following your regular assay procedure.

While the above program seems to present a rather large amount of work, it is hoped that each collaborator will complete it as far as possible. It would be best to carry out the complete study on the flour sample and, if time is then available, to do the same with the sample of enriched bread. Duplicate experiments would be very desirable but may be eliminated where the opportunity for doing this work is too limited.

It will be greatly appreciated if your results can be reported by the middle of March. Since it will take considerable time to organize the collaborators' data, reports received after the first of April will be difficult to include in the final report presented at the A.A.C.C. annual convention.

JOHN S. ANDREWS, Chairman  
Vitamin Assay Committee

### Results and Discussion

The data presented in Table I summarize the zeolite study carried out by examining the recovery of added thiamine. The first column shows the average fluorescence values due to the vitamin in the samples

TABLE I  
THIAMINE RECOVERY EXPERIMENTS

Sample	Fluorescence			Assay
	Flour (blank deducted)	1 $\mu$ g/g added B <sub>1</sub>	Blank	
	<i>Galv. units</i>	<i>Galv. units</i>	<i>Galv. units</i>	<i>mg/lb</i>
Enriched flour	28.8	6.17	6.3	2.11 <sup>1</sup>
Enriched bread	22.2	6.22	6.7	1.63
Pure thiamine	—	6.10	6.0	—

<sup>1</sup> Calculated for enriched flour: 2.09 mg/lb.

of enriched flour and bread. The second column shows the corresponding values for 1- $\mu$ g increments of added thiamine. It will be noted that these values for flour and bread are essentially identical. This indicates that, when passed through zeolite, both types of products have the same effect on the assay.

In order to determine the magnitude of this effect, the collaborators were requested to carry out recovery experiments using pure thiamine alone. The last value in the second column shows the result, 6.10 galvanometer units. This is slightly lower than those obtained from the flour and bread. Unfortunately, not all the collaborators reported results for the pure thiamine, and accordingly the value 6.10 is not strictly comparable with the others. If the comparison is confined only to those laboratories which carried out all three of the experiments,

the agreement is much closer. The values then become 6.10 for the flour, 6.08 for the bread, and 6.10 for the pure vitamin. The use of zeolite in the assay of thiamine does not appear to present any appreciable source of error.

The third column of the table shows the average blank values. It will be noted that they are approximately equivalent to 1  $\mu$ g of thiamine. There was, however, considerable variation between the individual collaborators. One laboratory reported extremely low values, while another obtained values which were correspondingly high. The high blank did not appear to have any adverse effect, however, since the assay result was almost identical with the average. The fact that the blank values for the flour and bread are somewhat

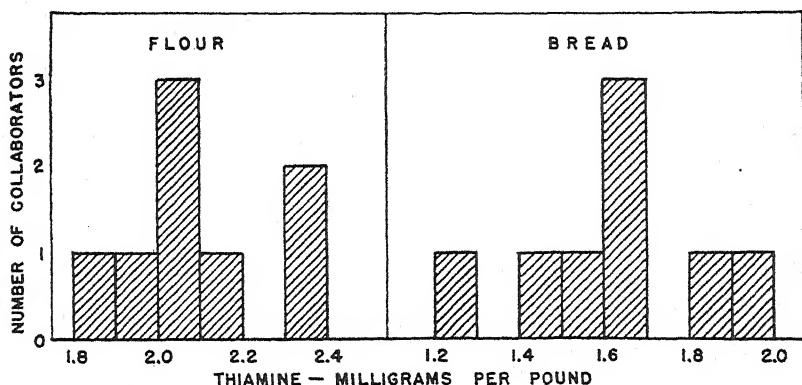


Fig. 1. Distribution of collaborators' thiamine assays using added thiamine for the standard.

higher than those for the pure thiamine indicates that the removal of interfering substances by the zeolite is not entirely complete. The greatest difference is seen in the sample of bread. However, in terms of the total fluorescence, this difference is quite small.

The last column shows the average assays for the two samples. The 2.11 mg/lb obtained for the flour very closely agrees with the calculated value, 2.09 (0.34 mg/lb native in the flour + 1.75 mg/lb of added thiamine). The value for bread is 23% lower.

The distribution of the individual collaborator's values is shown in Figure 1. Four, or 50%, of the values for the flour ranged between 2.0 and 2.2 mg/lb, *i.e.*, within  $\pm 5\%$ . Another collaborator was only slightly below this range (1.96 mg/lb). The lowest value was 1.82 mg/lb, and none of the data reveals the cause for this discrepancy. This collaborator obtained the same values for the added thiamine and the pure thiamine solution.

At least one of the two high values, ranging from 2.3 to 2.4 mg/lb,

can be attributed to an error in the standard. The submitted standard gave nearly 15% less fluorescence than this collaborator's U.S.P. thiamine. Use of the latter would have placed the assay within the  $\pm 5\%$  range. The other high value cannot be explained from the data. The collaborator did point out, however, that the actual work was carried out by an inexperienced technician.

The bread assays covered a somewhat greater range, 1.2 to 2.0 mg/lb. The values are based on the air-dried product, containing 9% to 10% moisture.

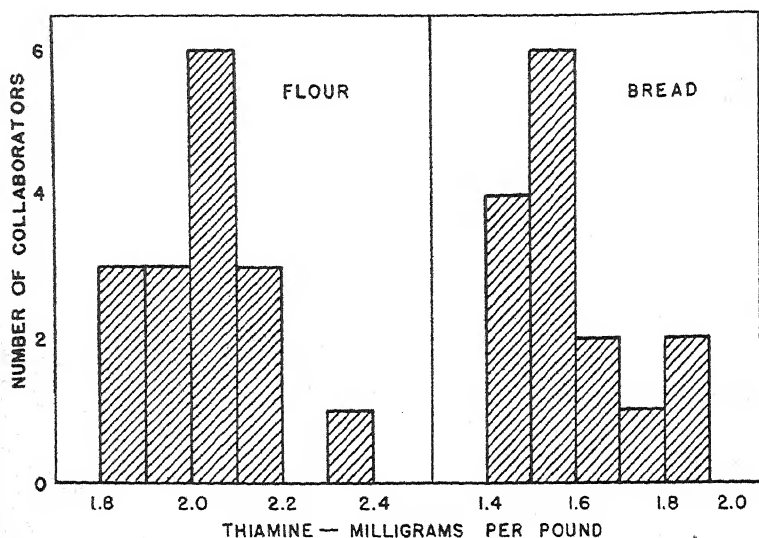


Fig. 2. Distribution of collaborators' thiamine assays using the regular thiochrome method.

Once again, 50% of the collaborators agreed within  $\pm 5\%$ . They did not all, however, represent the same laboratories which agreed on the flour. This makes it difficult to draw definite conclusions. For example, the inexperienced technician obtained an assay almost identical with the average. On the other hand, the lowest value for the bread was reported by a collaborator who obtained a flour assay within 2% of the calculated value. He attributes his low assay to an inadequate enzyme digestion period at too low a temperature. Digestion at 50°C for a longer period increased his value 11%, but still not enough to fall within the  $\pm 5\%$  range. The collaborator whose high assay of flour was attributed to discrepancies between standards was also high for the bread. The same correction would have brought his bread assay within the  $\pm 5\%$  range.

At the time the collaborators carried out the recovery experiments, they also assayed the samples by their regular procedures. In addition, eight other collaborators assayed the same samples. The results are summarized in Figure 2. The averages are very similar to those obtained from the recovery studies, 2.04 and 1.59 mg/lb for the flour and bread, respectively.

The highest value for the flour (2.38 mg/lb) came from another collaborator who reported discrepancies between the U.S.P. and submitted standards. In contrast to the previously mentioned discrepancy, the submitted standard was 10 to 15% *higher* than the U.S.P. preparation. This caused the assay by his regular procedure to be higher than that obtained from the recovery experiment. The fact that the latter was in excellent agreement with the calculated value suggests that the U.S.P. standard may have been in error, particularly since the same discrepancy was also obtained with the bread.

In both instances of the discrepancies between standards there is no proof about the correctness of one or the other. All other collaborators reported good agreement between their own and the submitted solutions of pure thiamine.

Another phase of the collaborative work involved a series of oxidation studies. Table II summarizes the effect of ferricyanide concen-

TABLE II  
EFFECT OF CONCENTRATION OF FERRICYANIDE AND ORDER OF  
ADDING OXIDIZING REAGENTS

Order of addition	Fluorescence	
	0.5% ferricyanide	4.0% ferricyanide
	<i>Galv. units</i>	<i>Galv. units</i>
Ferricyanide first	53	45
Alkali first	57	52
Mixed	54	51

tration and the order of adding the ferricyanide and alkali. It will be noted that the highest fluorescence values were obtained when the alkali was added first and followed immediately by the ferricyanide. This is in agreement with the observations reported by the English investigators, Wang and Harris (1942).<sup>2</sup>

The lowest results were obtained when the order was reversed and the ferricyanide added first. The use of the mixed reagent gave intermediate results.

In some laboratories these differences arising from the different order of adding the reagents were insignificant. In others they were

<sup>2</sup> Wang, Y. L., and Harris, L. J., Further notes on estimation of vitamin B<sub>1</sub> by the thiochrome method. *Chem. and Ind.* 61: 27-28, 1942.

quite marked. In only one instance did the addition of alkali first give a lower value, and this existed only at the lower concentration of ferricyanide. The 4% solution yielded the highest fluorescence values under the same conditions.

A preliminary study in the author's laboratory indicates that this effect of the order of adding the reagents is related to the concentration of ferricyanide. When the amount of oxidizing agent is kept low (one drop of 0.5% solution), either order yields practically the same result, provided the second addition is made immediately after the first. As the amount increases, however, the favorable effect of adding the alkali first is revealed. Under these conditions the fluorescence decreases only slightly in contrast to the much greater change which results when the ferricyanide is added first. This perhaps accounts for the observations made by Wang and Harris, since their procedure uses a considerable excess of ferricyanide.

TABLE III  
EFFECT OF CONCENTRATION OF FERRICYANIDE AND TIME OF SHAKING

Shaking time	Fluorescence	
	0.5% ferricyanide	4.0% ferricyanide
	<i>Galv. units</i>	<i>Galv. units</i>
Inverting	38	29
½ min	54	47
2 min	55	47
4 min	55	47

When adding alkali first, care should be taken to follow with the ferricyanide promptly. Otherwise decomposition of the thiamine occurs, resulting in decreased fluorescence. This does not occur if the ferricyanide is added first. No change results, even when the addition of alkali is considerably delayed.

It appears desirable to avoid an excess of ferricyanide, regardless of the order of adding the reagents. In the present collaborative study, the amount employed was only about one-fourth that recommended in *Cereal Laboratory Methods* (4th ed., 1941). In only one instance did this appear to be inadequate, and this failure was attributed to excessive quantities of iron in the zeolite.

The effect of ferricyanide concentration and shaking time is summarized in Table III. The majority of the collaborators reported maximum fluorescence values after one-half min of shaking. Relatively few required longer periods. It is interesting to note that, on the average, extended periods of shaking were without significant effect.

The final phase of the collaborative study considered the stability of the isobutanol extracts of thiochrome. How promptly must the fluorescence of these solutions be determined? Six of the collaborators prepared three isobutanol extracts each of the flour and bread samples. One set was measured promptly after preparation and the others after standing on the laboratory bench for 30 and 60 min, respectively. All but one of the collaborators found no difference after 30 min, and in most instances good stability was observed during the entire 60-min period.

### Summary

The thiochrome method as applied to the assay of thiamine in enriched flour and bread has been collaboratively studied. The use of zeolite apparently presents no appreciable source of error, since thiamine undergoes the base exchange similarly, both in pure solution and in cereal extracts. Assays of enriched flour yielded an average value (2.11 mg/lb) in very close agreement with that calculated from the assay of the unenriched flour and the added amount of pure thiamine (2.09 mg/lb). The average value for the bread made from the enriched flour was 23% below the flour value, suggesting that baking losses approximated this percentage figure. The possibility that some assay errors can be attributed to faulty thiamine standards is discussed.

The concentration of ferricyanide and the order of adding ferricyanide and alkali have some effect upon the assay. Particularly where a considerable excess of ferricyanide is employed, it is preferable to add the alkali first, followed immediately by the ferricyanide. One-half min shaking of the oxidation mixture resulted in the maximum fluorescence in most instances. Additional shaking has no significant effect. Isobutanol extracts of thiochrome appear to be sufficiently stable to present no problem in the fluorescence measurement.

### Acknowledgments

The author wishes to express his appreciation to Miss Marilyn Cooney for her able assistance in preparing this collaborative program and to the following individuals who participated in the collaborative study: A. W. Alcock, Western Canada Flour Mills Co., Ltd., Winnipeg, Manitoba; A. Arnold, Winthrop Chemical Co., Rensselaer, N. Y.; R. T. Bohn, General Baking Co., New York City, N. Y.; H. M. Boyd, General Mills, Inc., Minneapolis, Minn.; E. E. Brown, Anheuser-Busch, Inc., St. Louis, Missouri; F. J. G. de Leeuw, Lucidol Corp., Buffalo, N. Y.; D. E. Downs, Hollywood Candy Co., Centralia, Ill.; M. W. Mead, National Grain Yeast Corp., Belleville, N. J.; R. B. Meckel, American Inst. of Baking, Chicago, Ill.; B. L. Oser, Food Research Laboratories, Inc., Long Island City, New York; W. L. Rainey, Commander-Larabee Milling Co., Minneapolis, Minn.; W. Reeder, Campbell-Taggart Research Corp., Kansas City, Mo.; J. Rosin, Merck and Company, Inc., Rahway, N. J.; L. Rosner, Laboratory of Vitamin Technology, Chicago, Ill.; L. T. Saletan, Schwarz Laboratories, Inc., New York City, N. Y.; P. H. Towers, The Higginsville Flour Mills, Higginsville, Mo.; R. W. Truesdail, Truesdail Laboratories, Inc., Los Angeles, Cal.

## REPORT OF THE 1943-44 METHODS OF ANALYSIS SUBCOMMITTEE ON RIBOFLAVIN ASSAY<sup>1</sup>

JOHN S. ANDREWS, Chairman

General Mills, Inc., Research Department, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1944)

The first of the present series of collaborative studies on riboflavin assay methods was carried out two years ago (Andrews, 1943). The results showed that, despite considerable variations between the individual assays, microbiological and fluorometric methods yielded comparable values.

The collaborative study was continued the following year (Andrews, 1943a) with major emphasis on a fluorometric procedure. Once again wide variations were revealed, but there was evidence that under properly controlled conditions the method was capable of producing satisfactory results.

One major source of error appeared to lie in the use of "Florisil." While some laboratories found this adsorbent fairly efficient, others experienced difficulties. This was attributed to a lack of uniformity in the Florisil, and the analyst was cautioned to check its efficiency before using it for assay purposes. It was recommended that this be done by examining the recovery of riboflavin added to cereal extracts.

The second collaborative study also considered the use of permanganate for destroying interfering pigments. It was suggested that permanganate treatment might be omitted in the assay of enriched flour. Bread, however, appeared to require this oxidation since the use of Florisil alone would not prevent excessively high results.

The present collaborative study abandoned further attempts to examine complete assay procedures. Instead, its purpose was confined to an evaluation of permanganate and Florisil treatments when *separately* applied to the purification of cereal extracts. Recovery experiments involving enriched flour and bread were carried out by 13 collaborators and three types of assays reported. One showed the effect of omitting all purification and illustrated the influence of interfering substances in patent flour and bread derived therefrom. The second showed the extent to which these interfering substances were removed by permanganate oxidation. The third presented a similar picture as reflected by adsorption on Florisil. In addition, six other collaborators presented assays carried out by microbiological methods. These results have been compared with those obtained fluorometrically.

<sup>1</sup> Paper No. 60, Journal Series, General Mills, Inc., Research Department.

## The Collaborative Committee's Methods

A complete description of the recommended procedure, together with a detailed discussion of the reasons for its selection, is best afforded in the following communication addressed to the collaborators:

### TO THE MEMBERS OF THE A.A.C.C. COLLABORATIVE COMMITTEE ON RIBOFLAVIN ASSAY METHODS

A survey of the opinions of the collaborators on the subject of riboflavin assays reveals that the major problems in the assay procedures concern the use of permanganate and Florisil. Doubt is expressed about the resistance of riboflavin to permanganate oxidation and the quantitative aspects of the Florisil adsorption. In addition, more rapid methods are desired, together with the avoidance of pyridine.

The best type of collaborative study appears to be one which will attempt to answer some of these problems and evaluate the role of the oxidation and adsorption steps. Accordingly, a series of experiments have been designed and tested in the writer's laboratory. It is believed that their application in the present collaborative study will go far to develop a short, simple assay method.

Two ampules of riboflavin (5.54 mg in each, generously supplied by Dr. Arnold of Winthrop Chemical Co.) together with samples of enriched flour and bread are submitted for this work.

### Experimental

**Direct Reading.** Prepare the standard B<sub>2</sub> solution by dissolving contents of ampule (5.54 mg) in 554 g or ml of distilled water and dilute 10 ml of resulting solution to 100 ml (1  $\mu$ g/ml).

Weigh into 125 ml Erlenmeyer flasks six 1.5-g samples of enriched flour. Suspend uniformly in 50 ml of 0.1N H<sub>2</sub>SO<sub>4</sub>. Add the following amounts of standard B<sub>2</sub> (1  $\mu$ g/ml) solution: 0, 2, 4, 6, 8, and 10 ml, respectively.

Heat by autoclaving 15 min at 15 lb pressure, cool and adjust to pH = 4.3, using 10% NaOAc solution (requires approximately 6.3 ml). Transfer to 100 ml volumetric flask and make up to 100 ml with distilled water. Mix thoroughly and filter, discarding first approximate 10 ml filtrate. (Filtrate should be clear!) Measure fluorescence of aliquots (10 ml for Coleman and 15 ml for Pfaltz and Bauer fluorometers).

Prepare fresh, cold solution (in ice bath) of 5% hydrosulfite in 2% sodium bicarbonate and add 0.5 ml to each of above aliquots after the original fluorescence is determined. Mix quickly and determine fluorescence (blank).

Repeat the experiment, recording the duplicate values under B. (See Notes.)

Carry out the same study, using the sample of bread.

**KMnO<sub>4</sub> Treatment.** Take 25-ml aliquots of filtrates, either from the previous experiment or prepared anew, and add 0.2 ml 4% KMnO<sub>4</sub>. Mix and stand 1 min. Add 0.2 ml 3% H<sub>2</sub>O<sub>2</sub>. Transfer 10- or 15-ml aliquot to cuvette, depending on whether Coleman or Pfaltz and Bauer instrument is used, measure fluorescence and record on separate sheet, using same form as before (F<sub>orig</sub> in column I). Add 0.5 ml hydrosulfite solution, mix, and again measure fluorescence (F<sub>b1</sub> in column II).

**Florisil Treatment.** Take 20-ml aliquots of the filtrates and pass through the Florisil column (see *Report of Riboflavin Assay Committee*, Cereal Chemistry 20: 614, 1943, for use of this Florisil adsorption procedure). After washing and drying, elute the riboflavin with pyridine acetic acid solution, collecting 20 ml of eluate. Mix thoroughly.

Using 10- or 15-ml aliquots, measure fluorescence and record in column I. Add 0.5 ml hydrosulfite solution, mix, and measure F<sub>b1</sub> (Record in column II).

### Notes

It will be greatly appreciated if the collaborator will follow a standard form in reporting his results. This will greatly facilitate subsequent analyses of the data. The attached sample sheet will illustrate the form desired. On it is recorded a typical set of data.



## EXAMPLE OF REPORT SHEET

(Please follow this form carefully. This will greatly facilitate analysis of the collaborators' data.)

Sample	Test	Sample (i.e., enriched flour)						
		I F <sub>orig</sub>	II F <sub>bl</sub>	III F <sub>corr bl</sub>	IV F <sub>orig</sub> - F <sub>corr bl</sub>	V Avg.	VI F <sub>1</sub> $\mu$ g	VII F <sub>sample</sub>
1. 1.5 g flour	A	14.4	1.0	1.1	13.3	13.2	—	13.2
	B	14.2	1.0	1.1	13.1			
2. Plus 2 ml B <sub>2</sub> std.	A	19.5	1.0	1.1	18.4	19.0	2.9	13.0
	B	20.7	1.0	1.1	19.6			
3. Plus 4 ml B <sub>2</sub> std.	A	25.1	1.0	1.1	24.0	24.5	2.8	12.5
	B	26.2	1.1	1.2	25.0			
4. Plus 6 ml B <sub>2</sub> std.	A	32.8	1.0	1.1	31.7	31.8	3.6	13.8
	B	33.1	1.1	1.2	31.9			
5. Plus 8 ml B <sub>2</sub> std.	A	37.1	1.0	1.1	36.0	37.2	2.7	13.2
	B	39.5	1.0	1.1	38.4			
6. Plus 10 ml B <sub>2</sub> std.	A	44.3	1.0	1.1	43.2	43.5	3.2	13.5
	B	45.0	1.1	1.2	43.8			
Average							3.0	13.2

Final assay value A 2.9  $\mu$ g/g  
B

**Direct Reading.** Under the column marked "Test," A and B represent duplicate experiments. The column F<sub>orig</sub> refers to the fluorescence values of the riboflavin extracts, and F<sub>bl</sub> in column II, the values for the "blanks." Column III contains the corrected "blank" values. These are obtained by multiplying the "blank" readings in column II by a correction factor (1.05 if a 10-ml aliquot is used and 1.03 if the aliquot is 15 ml). These corrected "blank" values are then subtracted from the original fluorescence values in column I and the differences are recorded in column IV. The corrected values in column III should be calculated to the nearest tenth decimal place only, since greater accuracy is not justified in the average fluorometric reading.

The average for the duplicate values in column IV is recorded in column V. In column VI, please record the fluorescence values for each 1- $\mu$ g increment of added B<sub>2</sub>. Thus, in the attached table (column VI) the first recorded value, 2.9, is obtained by subtracting the 13.2 (column V) for the sample alone from that containing 2  $\mu$ g of added B<sub>2</sub> (19.0) and dividing by 2. The next value, 2.8, is the difference between the sample containing 2  $\mu$ g of added B<sub>2</sub> (19.0) and that to which 4  $\mu$ g of B<sub>2</sub> was added (24.5) divided by 2, etc.

In column VII, record the fluorescence values for the sample alone after deducting the fluorescence due to added riboflavin. These values are best calculated from the averages of duplicate experiments in column V and the final average (3.0) in column VI. The first value, 13.2, is, of course, the same as the corresponding value in column V, since it represents the extract with no added riboflavin. The next value, 13.0, is calculated by subtracting 6.0 (3.0, the average for 1  $\mu$ g in column VI, multiplied by 2  $\mu$ g) from 19.0 (column V); the next value, 12.5, by subtracting 12 (3.0  $\times$  4) from 24.5, etc. These values are then averaged, yielding, in the above example, 13.2, identical with the value obtained from the actual reading of the extract containing no added riboflavin (column V). It may happen, however, that these values are not identical, in which case the average of column VII should give the more accurate assay.

To calculate the assay value of the sample, use the final averages of columns VI and VII. Since the fluorescence of the sample is 13.2 and the fluorescence of 1  $\mu$ g of pure riboflavin under the same conditions is 3.0, the sample contains  $13.2 \div 3.0 = 4.4$   $\mu$ g. Since the sample weighed 1.5 g, it contains 2.9  $\mu$ g/g.

**KMnO<sub>4</sub> Treatment.** Two volume corrections are required here. The blank is corrected by multiplying by 1.05 or 1.03 as before and recording in column III. This corrected value is subtracted from F<sub>orig</sub> (column I) and the difference recorded in

column IV. Then, to correct for the added  $\text{KMnO}_4$  and  $\text{H}_2\text{O}_2$ , the values in column IV are multiplied by 1.016 and recorded in column IVa (not shown in example of report sheet). Actually, these corrections are very small relative to the accuracy of the usual fluorometer reading.

Data in columns V, VI, and VII are calculated the same as in previous experiment, using values in IVa rather than IV. Don't carry calculated values beyond tenth decimal place.

*Florisil Treatment.* Correct blank and record corrected values in column III. Subtract these values in column III from the corresponding values in column I and record the difference in column IV. Calculate values for V, VI, and VII as before.

While it is expected that some of the collaborators will be unable to carry out the complete program, it is hoped that all who can will do so. Carefully done work from many laboratories will go far in guiding the final selection of an accurate assay procedure. If, however, you feel that you can't complete the study outlined above, do as much as you are able.

Since a major purpose of the study is to determine the value of the  $\text{KMnO}_4$  and/or Florisil treatments, it will be more desirable to carry out part of each of the three experiments rather than to do one of them completely, if you find that some abbreviation of the program is desirable. It is suggested that, in this event, you omit the 2-, 6-, and 10- $\mu\text{g}$  increments of added riboflavin and even omit the duplicate determinations if necessary.

Please assay the flour and bread samples by the method you are now using and report with the other results.

In order to allow time to assemble and analyze all the collaborators' data and prepare a report for the forthcoming A. A. C. C. convention, your results should be sent in as soon as possible. It will be greatly appreciated if this is done before March 15. Data received after April 1 will be too late for formal inclusion in the convention report.

JOHN S. ANDREWS, Chairman  
Vitamin Assay Committee

A summary of the data obtained from recovery experiments on enriched flour is presented in Table I. The figures shown are the averages obtained when several increments of riboflavin were added to the cereal sample. The first column compares the fluorescence values

TABLE I  
RECOVERY EXPERIMENTS—RIBOFLAVIN ADDED TO ENRICHED FLOUR  
(Average values of 13 collaborators)

Treatment of extract	Fluorescence due to flour (blank deducted)	Fluorescence due to 1 $\mu\text{g/g}$ of added riboflavin	Assay result
	Galv. units	Galv. units	$\mu\text{g/g}$
None	9.13	2.18	2.78
Permanganate	9.18	2.21	2.75
Florisil	7.23	1.57	2.98

Calculated riboflavin content of sample: 2.76  $\mu\text{g/g}$

due to the sample alone. These were obtained by deducting the hydrosulfite blanks from the total fluorescence of the variously treated extracts. It will be noted that permanganate oxidation has no significant effect, since the values before and after this treatment are essentially the same. On the other hand, the value resulting from treatment with Florisil is significantly lower, suggesting that this adsorbent removes fluorescent material. Such a conclusion, however,

is hardly valid in view of a factor which prevents direct comparison with the preceding values. The fluorescence was determined on acidified pyridine extracts and under such conditions the fluorescence, at least of riboflavin, is depressed considerably.

The second column shows the extent to which fluorescence was increased by 1- $\mu$ g increments of added riboflavin. Once again it will be noted that treatment with permanganate had no significant effect. This observation leads to two conclusions. First, that permanganate had little, if any, value for the purification of extracts of patent flour, and second, that this oxidation did not result in destruction of riboflavin.

The value obtained after adsorption on Florisil is again low and is due not to any losses of riboflavin, but rather to the decreased fluorescence in the pyridine solution. Preliminary studies carried out in the writer's laboratory on pure riboflavin solutions indicated that this decrease amounted to about 25%. This would account for most of the differences noted in the tabulated values.

The last column shows the average assay results obtained from the recovery experiment. There is practically no difference between those obtained with and without permanganate. In both instances the assays are in excellent agreement with the calculated value, 2.76  $\mu$ g/g. The result obtained by treatment with Florisil is too high.

Figure 1 shows how the individual values were distributed. It is obvious that greatest uniformity resulted when no purification treatment was applied. With the exception of two collaborators, the reported assays were between 2.6 and 2.9  $\mu$ g/g. Introduction of permanganate oxidation, while not significantly changing the average, appeared to present difficulties resulting in somewhat poorer agreement between laboratories. A more thorough examination of this treatment appears desirable.

The results obtained by using Florisil were the most erratic. The values ranged between 2.2 and 3.6  $\mu$ g/g. It is unfortunate that a uniform supply of the adsorbent was not made available to all the collaborators. Had this been done, it might have been possible to determine the source of these discrepancies. At present it is not known whether the difficulty was due to the Florisil or to faulty technique in its application. There is increasing evidence that the former was at least partly at fault.

Perhaps the most significant result of these recovery experiments with enriched flour is the suggestion that relatively simple assay methods can be employed. Extraction with dilute acid, filtration, and measurement of the fluorescence appear to be all that is required. "Blanks" can readily be determined with hydrosulfite and reference

standards established with pure riboflavin. Whether the riboflavin can be used directly or should be added to the flour is not definitely known. However, this can be readily determined by a few comparison experiments.

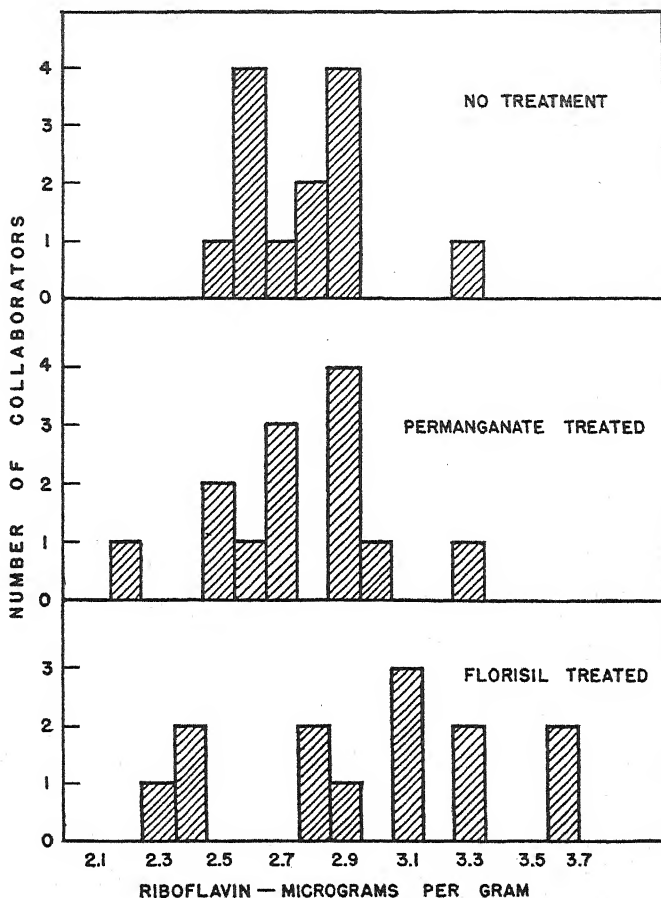


Fig. 1. Distribution of collaborators' riboflavin assays of enriched flour in which the flour extracts were subjected to different treatments.

A summary of the collaborative study on enriched bread is shown in Table II. These data demonstrate that bread presented a more complex problem than flour. The fluorescence values due to the bread alone indicate that permanganate treatment was beneficial. The decreased fluorescence resulting from this oxidation can be attributed to the removal of interfering impurities. This result is reflected in the values obtained for the 1- $\mu$ g increments of added riboflavin. The treatment resulted in an increase over that obtained directly on the

TABLE II  
RECOVERY EXPERIMENTS—RIBOFLAVIN ADDED TO ENRICHED BREAD  
(Average values of 13 collaborators)

Treatment of extract	Fluorescence due to bread (blank deducted)	Fluorescence due to 1 $\mu\text{g/g}$ of added riboflavin	Assay result
	Galv. units	Galv. units	$\mu\text{g/g}$
None	12.49	2.07	4.08
Permanganate	10.87	2.17	3.42
Florisil	9.34	1.53	4.08

Calculated riboflavin content of sample: 3.16  $\mu\text{g/g}$

extract and brought the value into excellent agreement with the 2.18 obtained on the sample of flour. The final assay results were also affected. The use of permanganate lowered the average assay more

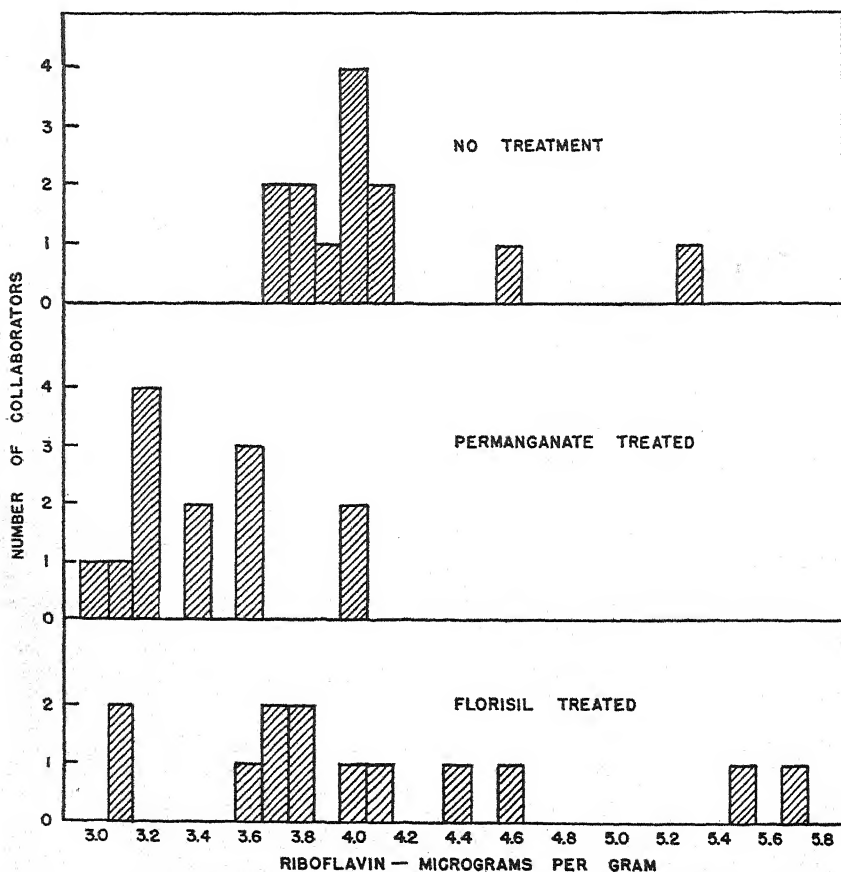


Fig. 2. Distribution of collaborators' riboflavin assays of enriched bread in which the bread extracts were subjected to different treatments.

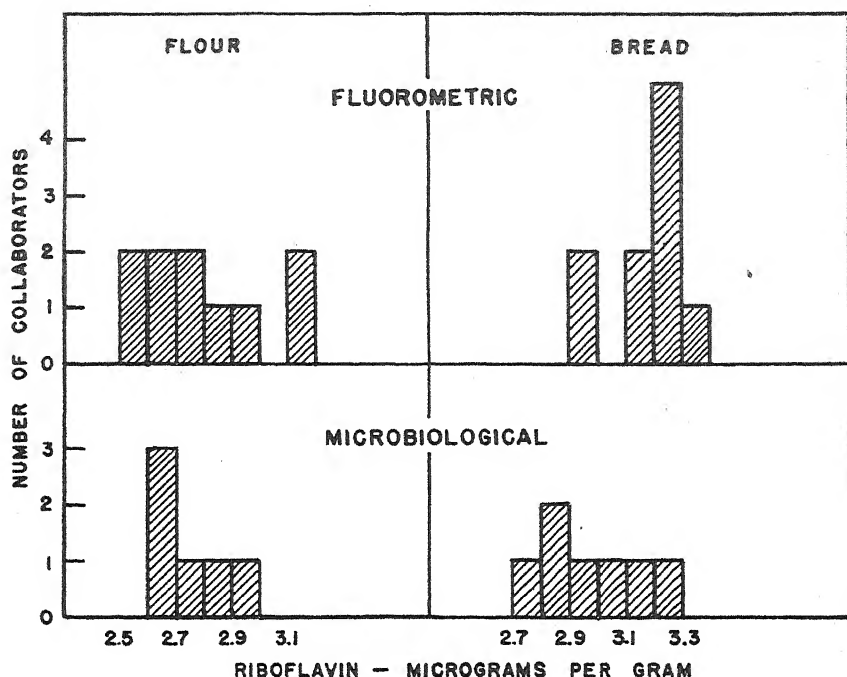


Fig. 3. Distribution of collaborators' riboflavin assays of flour and bread as determined by the fluorometric and microbiological procedures. The mean values are as follows:

		Riboflavin content	
		Fluorometric	Microbiological
		µg/g	µg/g
Flour		2.78	2.74
Bread		3.14	2.96

than 16% and brought it into much better agreement with the calculated value, 3.16 µg/g. This calculated value was based on the value for flour plus 0.42 µg/g added by the yeast. It is expressed on an air-dry basis (9.5% moisture).

As in the instance of the flour, the use of Florisil was of questionable value. The assay obtained by treatment with this adsorbent was excessively high and the same as that obtained directly on the extract. It should be remembered, however, that this experiment differed from the complete assay procedure. Florisil was used alone and not in conjunction with the permanganate. It can only be concluded that Florisil *when used alone* is not particularly effective.

The distribution of the individual collaborator's values is indicated in Figure 2. The results obtained by omitting purification treatment are quite uniform if the high values obtained by two collaborators are not considered. They range from 3.7 to 4.1 µg/g, or practically within  $\pm 5\%$ . Treatment with permanganate significantly lowered the as-

says, but contributed nothing to better uniformity. About half the collaborators were in close agreement with the calculated value, but the remainder tended to be too high. The results from the treatment with Florisil are too erratic to carry any significant meaning. Only two collaborators closely approached the calculated value. The others were widely scattered on the high side.

In addition to the recovery experiments, 16 collaborators reported fluorometric and microbiological assays carried out by their regular procedures. The results are summarized in Figure 3. They are, in general, quite satisfactory, as can be seen by the averages for the several assays.

For the flour, the fluorometric method yielded  $2.78 \mu\text{g/g}$ . The average obtained microbiologically was  $2.74 \mu\text{g/g}$ . These values compare very favorably with the calculated  $2.76 \mu\text{g/g}$ . The fluorometric value for the bread, 3.14, was equally in good agreement with that calculated from the bread ingredients,  $3.16 \mu\text{g/g}$ . The value obtained microbiologically was about 6% lower ( $2.96 \mu\text{g/g}$ ). The distribution of the individual values is shown graphically and represents a decided improvement over the results obtained from previous collaborative studies.

### Summary

The effect of permanganate and Florisil as employed separately in the fluorometric analysis of enriched flour and bread has been collaboratively studied. In the instance of enriched flour it appears that neither of these two purification treatments offers any value. Direct reading of flour extracts gave just as satisfactory a result as when either permanganate or Florisil were employed. Direct assay of the collaborative sample of enriched flour yielded a value of  $2.78 \mu\text{g/g}$ , in excellent agreement with the calculated value, 2.76. When the extracts were absorbed on Florisil and eluted on pyridine a somewhat higher value was obtained, indicating the inadequacies of the Florisil.

In the instance of enriched bread the use of permanganate aids considerably in removing interfering impurities. This treatment was not entirely effective, however, since the average value obtained was  $3.42 \mu\text{g/g}$ , as compared to the calculated value, 3.16. Treatment of the extract with Florisil had no effect on the final results, the same value being obtained as when the extracts were read directly.

Values reported by the collaborators employing their regular procedures were in general quite satisfactory. Both fluorometric and microbiological methods were represented and, particularly in the instance of flour, yielded values which closely agreed with the calculated values. Fluorometric assays of enriched bread were somewhat higher

than those obtained microbiologically and were in closer agreement with the value calculated from the bread ingredients.

#### Acknowledgments

The author wishes to express his appreciation to Miss Jane Spence for her able assistance in preparing this collaborative program and to the following individuals who participated in the collaborative study: H. Boeddeker, General Mills, Inc., Minneapolis, Minn.; H. M. Boyd, General Mills, Inc., Minneapolis, Minn.; W. Brew, Ralston Purina Co., Inc., St. Louis, Mo.; R. T. Conner, General Foods Corp., Hoboken, N. J.; C. N. Frey, Fleischmann Laboratories, New York City, N. Y.; D. Glick, Russell-Miller Milling Co., Minneapolis, Minn.; C. G. Harrel, Pillsbury Flour Mills Co., Minneapolis, Minn.; L. C. Norris, Cornell University, Ithaca, N. Y.; L. W. Haas, The W. E. Long Company, Chicago, Ill.; S. H. Rubin, Hoffmann-LaRoche, Inc., Nutley, N. J.; R. A. Stewart, Quaker Oats Company, Chicago, Ill.; H. B. Wigman, Mellon Institute, Pittsburgh, Penn.; V. O. Wodicka, Chicago Quartermaster Depot, Chicago, Ill.

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## REPORT OF THE 1943-44 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

FRANK R. SCHWAIN, Chairman

Kroger Grocery and Baking Co., Columbus, Ohio

(Read at the Annual Meeting, May 1944)

There is a definite need for a reliable, sensitive cooky-baking test to supplement analytical data, particularly the viscosity test, in evaluating cooky flours for specific baking applications. For the last three years the committee has been developing a test-bake formula for cooky flours which would differentiate such flours satisfactorily. As an outgrowth of these studies it became apparent that spread potentialities may vary with the white wheat varieties from different localities (Hanson, 1943). There were also indications that granulation in itself can change the spreading properties of a given flour (Loving, 1942; Hanson, 1943a). If this phenomenon could be manifested in practice, granulation might well be added to cooky flour specifications.

This year, then, the committee decided to attempt to translate the predictions of its cooky test into actual performances in bakeries. In addition it planned to determine if possible the practical differences in performance of flours from different areas or of various granulations. In order to accomplish this most efficiently, the work was so allocated



as to place specific tasks with those individuals whose everyday duties were closely allied to similar assignments. Flour preparations and complete analyses were in the main referred to mill-chemist members. Cooky baking tests and subsequent flour classifications were assigned to three different testing laboratories. Shop performances were allotted to those committeemen actively connected with four different cooky bakeries. It was felt that the findings then should command the interest of a larger portion of our membership.

Because of the acute shortage of Michigan wheat this year, the original plan to utilize most of the white wheat varieties, which had been considered by previous committees, had to be abandoned, and Pacific Coast wheat flours of three different granulations were studied. Analytical data for the flours, which were all unbleached and of 100% extraction, are given in Table I. Flours A and B came from the

TABLE I  
ANALYSES OF TEST FLOURS

Flour <sup>1</sup>	Ash (15% mb)	Protein (15% mb)	Apparent viscosity			Maltose <sup>2</sup> (Approximate)
			No-time	1 hr digestion	2 g protein	
	%	%	<sup>°</sup> MacM	<sup>°</sup> MacM	<sup>°</sup> MacM	
A	0.40	7.65	35	48	66	221
B	0.37	7.85	39	56	74	209
C	0.41	7.70	36	53	74	203

<sup>1</sup> Flours A and B from same wheat mix differing only in grinding and bolting operations in milling.

<sup>2</sup> Maltose reported from Pressuremeter results (Conversion factor 1.7).

same wheat mix, but by means of changes in grinding and bolting operations, a difference in granulation was secured. Flour C was supplied by another mill. The flours are quite similar in composition. With granulation given the prime consideration, however, this factor was determined by two methods. The first method was that followed by the last two committees and consisted of measuring the "throughs" of a Ro-Tap testing sieve shaker fitted with a 250-mesh wire screen. The second method was another Ro-Tap test in which the main fractions were separated by a Ro-Tap clothed with five silks of different mesh. The results are given in Table II.

There is less difference in granulation in the test flours this year than formerly. Previous committees have worked with Michigan flours which differed as much as 11% in the "throughs" of a 250-mesh wire. Coast wheat flours have usually been the coarsest of all. This time the simplified method showed a differential of only about 6% between the coarse-ground and fine-ground products. The relatively narrow spread in granulation between flours A and B was attributed

TABLE II  
RO-TAP GRANULATION STUDIES OF TEST FLOURS

Flour	Wire screen study through 250-mesh <sup>1</sup>	Silk sieve classification					
		Over 11 XX	Over 12 XX	Over 13 XX	Over 14 XX	Over 15 XX	Through 15 XX <sup>2</sup>
A	%	%	%	%	%	%	%
B	86	3.9	22.1	32.4	27.2	11.8	2.6
C	91	3.1	20.0	27.2	25.1	18.3	6.3
	85	3.6	19.0	23.8	28.5	21.4	3.7

<sup>1</sup> 250-mesh = 25 XX = 0.0024" opening.

<sup>2</sup> 15 XX silk = 170-mesh = 0.0037" opening.

by the mill to the quality of the Pacific Coast crop which required less tempering water to secure the flour desired, with the result that the middlings pulverized more readily than expected. The more comprehensive study employing silks would place flour C intermediate in granulation between A and B.

Again, the same laboratory procedure and test-bake formula described by Hanson (1943) were followed. The W/T or spread factors secured by the three collaborating laboratories are shown in Table III.

TABLE III  
LABORATORY SPREAD FACTORS (W/T) OF FLOUR SERIES

Flour	I	Collaborator II	III
A	8.5	8.2	6.8
B	8.3	8.1	6.8
C	9.1	9.1	7.3

Without exception, flour C exhibited the best spread, with both A and B close behind. Although the coarser flour (A) appeared slightly better than the finer-ground, the advantage was not nearly so marked as in last year's Michigan flours, for example, when there was a greater difference in granulation.

Regular shop tests were also made in four different bakeries, utilizing formulas in current use and under normal operating conditions. These tests comprise Band and Reel oven performances. Three distinct types of products were made by the bakeries—wire cut cookies, rotary goods, and cutting pieces. Observations on spreading properties were determined 30 min out of the ovens. Table IV shows the results secured.

In the case of wire cut cookies, flour C performed the best in all four shops. Neither A nor B worked as well, both rating about the

TABLE IV  
SHOP SPREAD FACTORS (W/T) OF FLOUR SERIES

Flour	Collaborator					
	IV (Band oven)	V (Reel oven)	VI (Reel oven)	VII (Reel oven)		
WIRE CUT COOKIES						
A	3.2	7.8 <sup>1</sup>	5.5 <sup>1</sup>	7.0	3.6	4.1
B	3.2	8.0 <sup>1</sup>	5.7 <sup>1</sup>	7.2	3.8	4.0
C	3.5	8.2	5.9	7.9	4.5	4.4
ROTARY GOODS						
A	7.5	5.9	8.4 <sup>2</sup>	(Band oven)		
B	7.5	5.8	7.6 <sup>2</sup>	7.8	10.1	
C	7.4	6.1	7.4 <sup>2</sup>	8.0	10.2	
CUTTING PIECES						
A	6.9	10.4		(Band oven)		
B	6.5	10.4		8.2 <sup>2</sup>		
C	6.7	10.8		7.8 <sup>2</sup>		
				8.3 <sup>2</sup>		

<sup>1</sup> This collaborator added 2.5 lb sugar per 100 lb flour to flours A and B—wire cut only.

<sup>2</sup> Formula used "lean"; all others "medium."

Note—Where collaborator tried several kinds of wire cut or rotary cookies the results are listed side by side.

same. This is in fairly good agreement with the laboratory findings. Collaborator V deliberately incorporated 2.5 lb additional sugar per 100 lb of flour for both A and B wire cut doughs, but did not secure as good a spread in either case as that shown by the flour C dough without additional sugar.

Rotary goods, however, appeared less discriminative as to flour type.

TABLE V  
CHECKING STUDIES OF FLOUR SERIES

Flour	Number of checked cookies in 100 after 24 hr			
	Collaborator V	Collaborator VII		
		Lot 1	Lot 2	Lot 3
A	17	33	24	22
B	29	26	19	67
C	12	7	17	6

Cutting pieces, on the other hand, favored flour C in two out of three shops with an indication of poorer performance on the part of the finer-ground flour (B). This compares favorably with laboratory predictions.

Checking or evidence of cracking was also considered. Each bakery was asked to determine the number of cookies per hundred, which showed signs of checking 24 hr out of the oven. Two shops experienced no checking. The observations made by the other two plants on their more brittle types of goods are summarized in Table V.

In these frangible pieces, far less checking was observed from flour C than from either A or B. This bears reinvestigation. The evidence presented is too limited to draw any accurate conclusions, though milling practices may have some bearing.

### Conclusions and Recommendations

It would appear, from the data presented thus far, that the current laboratory test formula devised by earlier committees has application where flours intended for wire cut or sheeter cooky production are concerned. However, for rotary goods, the test appears to have less value and the results should be viewed with some hesitancy in evaluating flours for this purpose. This is logical in view of the larger amount of water usually required for sheeter and wire cut goods which compares more favorably with the water content of the laboratory test formula.

There are also indications with wire cut goods and sheeter cookies that milling technique may be a factor in explaining differences in performance in spite of similar wheat types and analyses. The phenomenon of checking should not be overlooked from the same standpoint.

Coarse-ground flour may exhibit better spreading characteristics than the same flour finely ground, if there is a more significant difference in granulation than that experienced here. Further experimentation should answer this point.

The committee suggests that the present laboratory test formula appears to be adaptable for differentiating flours intended for wire cut and sheeter production. For rotary purposes, a new formula may have to be developed which requires far less water; for example, 6 or 7%. Perhaps the answer lies in some sort of laboratory-size die for cutting out the cookies under pressure. This would more nearly approximate practical conditions.

The committee recommends additional studies of the importance of granulation in relation to the quality of cooky flours. The two

methods of measuring granulation employed here should be further investigated to determine which has the greater practical significance.

Correlating laboratory results with shop tests is not only desirable but necessary.

Flours from the white wheats of such areas as New York, Michigan, and the Pacific Coast certainly bear further consideration to determine whether the differences noted in previous laboratory tests are manifested from crop to crop and are actually demonstrable in commercial production.

#### Acknowledgments

The chairman wishes to express his appreciation of the excellent cooperation accorded him by the committee which included Miss Pearl Brown, W. H. Hanson, T. E. Hollingshead, Jan Micka, H. M. Simmons, O. P. Skaer, and L. S. Thomson. Acknowledgment is also made of the work done by P. W. Hodler. Appreciation is due Henkel Flour Mills, Centennial Flouring Mills, Perfection Biscuit Company, Kroger Grocery and Baking Company (Columbus Unit), and the Sawyer and Strietmann Divisions of the United Biscuit Company of America for their individual contributions.

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## REPORT OF THE 1943-44 METHODS OF ANALYSIS SUBCOMMITTEE ON THE DETERMINATION OF IRON IN CEREAL PRODUCTS

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(Read at the Annual Meeting, May 1944)

The lack of agreement in the determination of iron has long been apparent from previous collaborative studies. This could be due, among other things, to the preparation of the ash for the determination of iron or to the contamination of iron from dishes and tongs. There is the possibility of nonuniformity of mixing of the enriched flour, but in this report the variation on the unenriched flour was as great as on the enriched. Iron can easily become a source of contamination, but this would not account for the very low results so often encountered. Such results could be explained by the presence of pyrophosphate in the ash, since the presence of pyrophosphate affects the color develop-

ment and must be converted to orthophosphate. Iron contamination from dishes used for ashing can be a serious problem; therefore, platinum or glass dishes are the most suitable. The dishes should be cleaned by dipping them successively in two or three boiling hydrochloric acid solutions, and cleaning should be continued until a blank determination carried out on the dish alone gives a negligible coloration. Large blanks on platinum dishes have been found if they were previously used for fat determinations, and just one boiling with hydrochloric acid will not remove all the contaminating iron present. Glass or platinum tongs should be used to handle the crucibles.

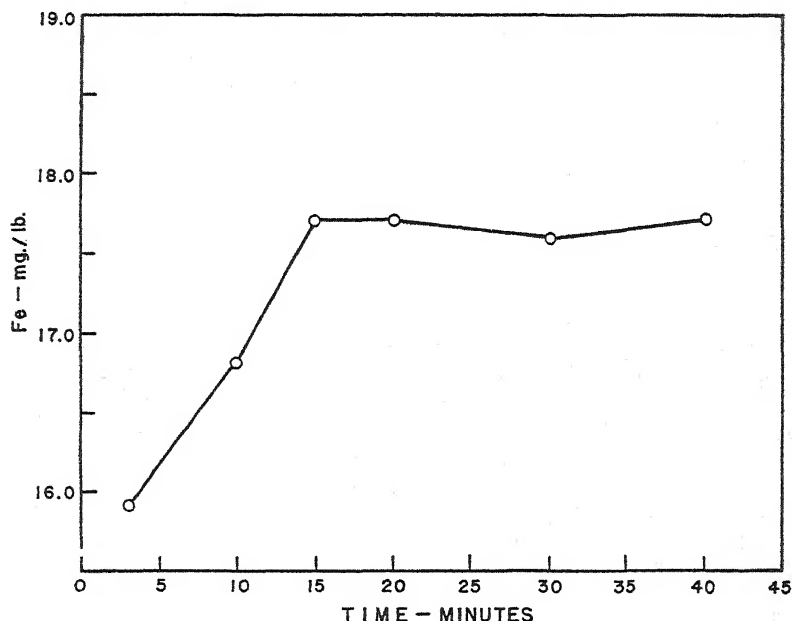


Fig. 1. Influence of time of digestion of ash from whole wheat flour with boiling hydrochloric acid solution (1 : 1) on the apparent iron content of the flour.

Preparatory to sending out the samples for collaborative work, a study was made of the heating period necessary to convert the pyrophosphate to the orthophosphate in the ash of whole wheat flour. The result of this study is shown in Figure 1. The time of heating was varied from 3 min to 40 min. The minimum time of heating required to secure the maximum iron value was found to be 15 min; however, 30 min was specified in the method sent to the collaborators to insure a reasonable safety factor.

Figure 2 shows a spectral transmission curve for the  $\alpha, \alpha'$ -dipyridyl complex. This was obtained with a Beckman Spectrophotometer.

The maximum absorption band was found to be at 520  $m\mu$ . The collaborators, therefore, were instructed to use an appropriate filter for this range.

In a continuation of the work of the 1942-43 Subcommittee on Methods of Analysis of Iron (Howe, 1943), the Committee undertook

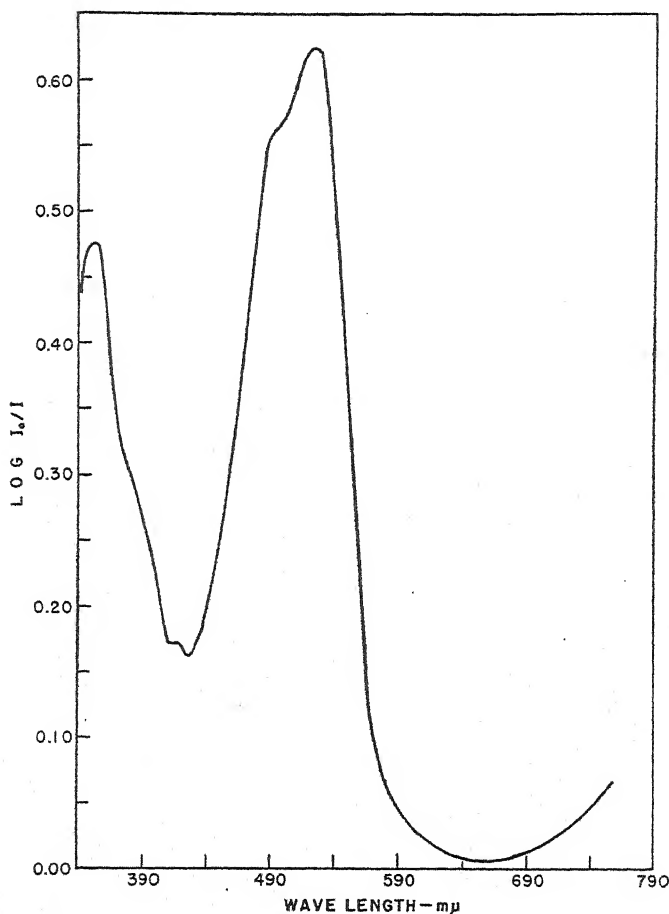


Fig. 2. Spectral distribution curve for the  $\alpha, \alpha'$ -dipyridyl complex (prepared from a standard iron solution containing 10  $\mu\text{g}$  Fe per ml).

a study of the preparation of cereal products by various methods including hydrochloric acid treatment of the ash, sodium carbonate fusion, and wet digestion preparatory to the determination of iron by the  $\alpha, \alpha'$ -dipyridyl method. This study was made to ascertain the validity of dry ashing of cereal products and to compare the numerous methods now employed in various laboratories as routine determina-

tions with the  $\alpha, \alpha'$ -dipyridyl method. The samples sent to the collaborators comprised (1) an unenriched flour; (2) the same flour enriched with 10 mg of iron as ferrum reductum; (3) a bread made from the enriched flour; (4) a whole wheat flour; and (5) a bread made from the whole wheat flour.

The samples were prepared with a great deal of care, and the flour enriched with ferrum reductum was thoroughly mixed; in spite of this fact, several collaborators stated that the sample was not uniform. The whole wheat flour was passed over a magnet to remove any small pieces of metal which might have been present from the milling rolls, as several laboratories have reported finding such small pieces of metal in previous samples of whole wheat flour. The bread was baked, using an average formula, air dried, and ground in a mortar.

The three methods of preparation were as follows: (1) The dry ashing was accomplished by ashing the products at 550–600°C overnight, taking the ash up in 2 ml of HCl, heating for 30 min, filtering, and making up to 100 ml; (2) for the sodium carbonate fusion, the samples were ashed in the usual manner, then mixed with 0.5 g of purified sodium carbonate, and fused. The melt was taken up in 5 ml of 1 : 1 HCl, heated for 30 min, filtered, and made up to volume. (3) The wet ashing procedure was that of Jackson (1938). This method is time-consuming and was not employed with the intention of using it as a routine method, but because the Committee wanted to compare the results of dry versus wet ashing. In our laboratory, a loss of iron upon dry ashing of cereal products has never been observed, and this fact has been substantiated by other collaborators (Andrews and Felt, 1941). The collaborators were also requested to send in the results as determined by the method used routinely in their own laboratories; these included the use of ortho-phenanthroline, potassium thiocyanate, and numerous variations of the  $\alpha, \alpha'$ -dipyridyl method. The summarized results are given in Tables I–VI.

The following conclusions are evident:

Wet ashing did not consistently give higher results than dry ashing.

There was no particular advantage in the sodium carbonate fusion.

Individual laboratory methods did not give any closer agreement than the method suggested by the Committee.

The use of the  $\alpha, \alpha'$ -dipyridyl method with dry ashing is as satisfactory a method as is now available for the determination of iron in cereal products. More practice and improvement in technique should bring better agreement between laboratories.



TABLE I  
IRON CONTENT OF UNENRICHED FLOUR  
Iron content as Fe in mg/lb ("as is" moisture basis—12.6%)

Collaborator number	Hydrochloric acid treatment	Sodium carbonate fusion	Wet digestion	Individual laboratory method
1	4.0	4.3	4.0	—
2	3.6	3.6	—	—
3	2.8	3.1	—	4.1
4	—	—	—	5.0
6	3.9	3.9	5.7	3.9
7	4.0	4.1	4.2	—
8	4.3	4.4	4.4	4.3
10	3.6	3.8	3.6	4.2
11	4.0	4.0	—	4.1
12	3.9	3.5	—	—
13	3.3	4.2	—	—
14	4.4	5.1	—	—
15	3.8	3.5	3.8	—
16	—	—	—	4.4
17	—	—	—	3.8
Maximum	4.4	5.1	5.7	5.0
Minimum	2.8	3.1	3.6	3.8
Mean	3.8	3.9	4.3	4.2
Standard deviation	0.434	0.523	0.749	0.369

TABLE II  
IRON CONTENT OF FLOUR ENRICHED WITH 10 MG/LB OF IRON AS  
FERRUM REDUCTUM  
Iron content as Fe in mg/lb ("as is" moisture basis—12.6%)

Collaborator number	Hydrochloric acid treatment	Sodium carbonate fusion	Wet digestion	Individual laboratory method
1	17.2	17.6	18.6	—
2	12.7	12.3	—	—
3	10.0	12.0	—	13.3
4	—	—	—	16.5
6	13.6	14.2	16.8	13.2
7	12.4	12.0	12.1	—
8	15.8	16.8	13.5	13.9
10	12.8	13.8	13.3	13.9
11	12.3	12.4	—	12.3
12	13.0	13.6	—	—
13	10.2	14.8	—	—
14	13.8	16.2	—	—
15	14.6	15.0	14.8	—
16	—	—	—	18.1
17	—	—	—	13.9
Maximum	17.2	17.6	18.6	18.1
Minimum	10.0	12.0	12.1	12.3
Mean	13.2	14.2	14.8	14.4
Standard deviation	2.05	1.92	2.43	1.92

TABLE III  
IRON CONTENT OF BREAD BAKED FROM ENRICHED FLOUR  
Iron content as Fe in mg/lb ("as is" moisture basis—10.6%)

Collaborator number	Hydrochloric acid treatment	Sodium carbonate fusion	Wet digestion	Individual laboratory method
1	17.2	16.7	18.5	—
2	14.5	14.3	—	—
3	12.4	12.6	—	16.5
4	—	—	—	16.7
6	16.2	16.3	16.2	16.3
7	16.5	15.4	16.4	—
8	15.6	16.0	15.1	15.2
10	16.2	16.3	15.6	15.3
11	11.8	14.1	—	15.0
12	16.3	16.3	—	—
13	13.8	15.2	—	—
14	17.8	18.5	—	—
15	15.8	15.7	15.9	—
16	—	—	—	20.6
17	—	—	—	14.9
Maximum	17.8	18.5	18.5	20.6
Minimum	11.8	12.6	15.1	14.9
Mean	15.3	15.6	16.3	16.3
Standard deviation	1.85	1.49	1.18	1.87

TABLE IV  
IRON CONTENT OF WHOLE WHEAT FLOUR  
Iron content as Fe in mg/lb ("as is" moisture basis—12.6%)

Collaborator number	Hydrochloric acid treatment	Sodium carbonate fusion	Wet digestion	Individual laboratory method
1	18.9	18.1	18.4	—
2	16.1	14.8	—	—
3	14.0	13.3	—	17.2
4	—	—	—	18.5
6	16.3	16.3	14.3	16.7
7	16.4	16.0	18.1	—
8	17.0	17.5	16.6	16.3
10	18.6	16.7	18.0	16.0
11	14.5	18.2	—	19.1
12	18.5	17.0	—	—
13	16.3	14.1	—	—
14	19.0	18.6	—	—
15	17.6	17.5	17.0	—
16	—	—	—	22.7
17	—	—	—	16.9
Maximum	19.0	18.6	18.4	22.7
Minimum	14.0	13.3	14.3	16.0
Mean	16.9	16.5	17.1	17.9
Standard deviation	1.65	1.69	1.52	2.20

TABLE V  
IRON CONTENT OF BREAD BAKED FROM WHOLE WHEAT FLOUR  
Iron content as Fe in mg/lb ("as is" moisture basis—12.8%)

Collaborator number	H <sub>2</sub> drochloric acid treatment	Sodium carbonate fusion	Wet digestion	Individual laboratory method
1	17.2	18.1	17.8	—
2	18.8	19.4	—	—
3	14.6	15.2	—	19.5
4	—	—	—	19.9
6	20.7	19.9	18.2	20.7
7	20.2	20.9	20.5	—
8	19.6	19.1	18.9	19.8
10	20.9	20.7	21.7	18.9
11	18.6	20.4	—	20.9
12	20.1	20.4	—	—
13	18.4	19.7	—	—
14	21.4	23.8	—	—
15	20.4	20.0	19.4	—
16	—	—	—	25.1
17	—	—	—	17.9
Maximum	21.4	23.8	21.7	25.1
Minimum	14.6	15.2	17.8	17.9
Mean	19.2	19.6	19.4	20.3
Standard deviation	1.90	1.99	1.47	2.15

TABLE VI  
AVERAGE RESULTS OF THE COLLABORATORS WHO EMPLOYED ALL  
THREE METHODS  
Iron content as Fe in mg/lb ("as is" moisture basis)

Sample	H <sub>2</sub> drochloric acid treatment	Sodium carbonate fusion	Wet digestion
(1) Patent flour	3.9	4.0	4.4
(2) Enriched patent flour	14.4	14.9	14.9
(3) Bread made from enriched patent flour	16.3	16.1	16.3
(4) Whole wheat flour	17.4	17.0	17.1
(5) Bread made from whole wheat flour	19.8	19.8	19.4

#### Acknowledgment

The members who collaborated in this study were A. Wahl, E. F. Budde, Joseph Rosin, Aaron Arnold, John Andrews, C. G. Harrel, C. N. Frey, Wendell Reeder, Charles Hoffman, M. H. Neustadt, Morris Mead, Oscar Skovholt, Rosaltha Sanders, John B. Thompson, Jr., and Clarence Felt.

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# REPORT OF 1943-44 COMMITTEE ON SELF-RISING AND PHOSPHATED FLOURS

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The formula as given in the Biscuit Test for Self-rising Flour (Cereal Laboratory Methods, 4th ed., 1941, p. 122) is based on soft wheat flour and the work of this year's committee was the adaptation of this test to hard wheat flour.

Hard wheat flours differ from soft wheat flours in that they require more water to make a soft dough and do not give as tender a biscuit. The problem then was to determine the proper absorption to use and whether the amount of shortening should be increased. The flours chosen for this test were a soft wheat flour and a hard wheat flour, both of family grade, the soft wheat flour to be used as the standard. These were about 80% patents and analyzed as follows:

	Soft wheat flour	Hard wheat flour
Moisture	15.0%	15.0%
Ash	0.36%	0.46%
Protein	8.1%	10.0%
pH	5.1	6.0

The flours were plain, and each collaborator was asked to make them into self-rising flour, using either of the following formulas:

	Monocalcium phosphate Hydrated g	Anhydrous g
Flour (15% moisture basis)	227.7	227.70
Sodium bicarbonate	3.4	2.85
Phosphate	4.3	3.42
Flour salt	4.6	4.69

Biscuits were baked from the self-rising soft wheat flour, following the formula and procedure given in the aforementioned Biscuit Test for Self-rising Flour. These biscuits were used as the reference against which the other biscuits were scored.

The hard wheat self-rising flours were baked using 12.5, 15.0, and 17.5% shortening (basis self-rising flour at 15% moisture). As the shortening is increased, it is necessary to decrease somewhat the amount of water used. Preliminary bakings showed that for each 2.5% increase in shortening the absorption should be decreased 1%. This agrees with the data of Schwain and Loving.<sup>1</sup> These preliminary bakings also indicated that the absorption of this hard wheat self-rising flour (15% moisture and 12.5% shortening) was 66.6%. Several of the collaborators found this to be about 1% too high.

<sup>1</sup> Schwain, F. R., and Loving, H. J., 1944. The shortener tolerance of biscuit and self-rising flours. Cereal Chem. 21: 27-32.

Six members of the committee collaborated on the bakings and the average scores are shown in Table I. The variations in the individual scores were so great that the averages shown in the table mean very little. However, from the conclusions reached by each member, definite recommendations can be made. Four of the collaborators

TABLE I  
EFFECT OF INCREASING FAT IN BISCUIT FORMULA USING  
HARD WINTER WHEAT FLOUR<sup>1</sup>

Factor	Perfect score	Scores of biscuits made by six collaborators					
		12.5% fat		15% fat		17.5% fat	
		Average	Range	Average	Range	Average	Range
Grain	10	8.4	7-10	9.0	7.7-10	9.1	8.0-10
Tenderness	10	8.3	7.7-9.0	9.1	8.7-9.4	9.5	9.0-9.7
Flavor	20	19.0	18.0-20.0	19.3	18.5-20.0	18.8	17.0-20.0
Color	20	17.4	15.0-19.1	17.8	15.7-19.5	17.5	16.0-19.3
Volume	40	40.6	38.0-43.3	39.8	39.0-41.4	38.6	34.0-43.1
Total	100	93.8	90.2-96.2	95.0	91.4-98.8	93.4	89-97.8
pH		7.08	6.71-7.28	7.08	6.71-7.27	7.15	6.71-7.27

<sup>1</sup> Cereal Laboratory Methods, 4th ed., 1941.

Four of the collaborators preferred the biscuits made with 15% shortening, while two preferred those made with 17.5% shortening.

thought that 15% shortening gave optimum results, while the other two thought that 17.5% fat could be used. Two of the four who got the best results with 15% fat got definitely poorer biscuits when the amount was increased to 17.5%. Absorption values checked fairly well, varying from 62.5% to 65.6% where 15% shortening was used and the self-rising flour moisture was 15%.

On the basis of these conclusions the committee recommends the following formula for the Biscuit Test for Self-rising Flour made with hard winter wheat flour:

Ingredients	Grams	Percentage based on flour	Percentage composition
Flour, hard winter self-rising (15% moisture basis)	240	100	55.6
Shortening, hydrogenated (25°C)	36	15.0	8.4
Water, distilled	155	64.6	36.0
Totals	431		100.0

#### Acknowledgments

The members who collaborated in this study were: R. A. Barackman, V. E. Fisher, H. R. Goforth, Elizabeth McKim, R. M. McKinstrie, W. C. Meyer, and Elmer Modeer.

# NUTRIENT CONTENT OF ALCOHOL FERMENTATION BY-PRODUCTS FROM VARIOUS GRAINS

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(Received for publication June 5, 1944)

The nutrient content of alcohol fermentation by-products from yellow corn grain mixtures as produced under commercial conditions has been determined recently by Bauernfeind, Garey, Baumgarten, Stone, and Boruff (1944). These corn grain mash bills (formulae) are favored for the production of whiskey or alcohol, and the recovery of by-products from them has been in process for many years. During the past year certain sections of the country have experienced an apparent corn shortage, and the distilling industry has been forced to use other grains such as wheat, granular wheat flour, and rye. The use of these other grains has raised some questions as to the composition and nutritive value of by-products produced from them. It is the purpose of this paper to present data on the nutrient content of these by-products prepared with pilot plant equipment.

## Experimental Fermentations

A weighed quantity of corn, wheat, granular wheat flour, or rye was cooked in pilot plant equipment at maximum temperatures of 155°, 212°, 280°, and 312°F (68.3°, 100.0°, 137.8°, and 155.6°C) respectively. The amounts of water used per bushel of grain were as follows: 20 gal for corn cooked at any of the specified temperatures, and for wheat and granular wheat flour cooked at 155°F (68.3°C); 13.8 gal for wheat and granular wheat flour cooked at each of the specified temperatures above 155°F and for rye cooked at 280°F (137.8°C); and 22 gals for rye cooked at 155°F (68.3°C). After cooking and cooling to malting temperature, 140–146°F (60.0–63.3°C), barley malt was added for the purpose of conversion. The mash bills for this report were (1) wheat 92%, barley malt 8%; (2) granular wheat flour 92%, barley malt 8%; (3) rye 90.9% and barley malt 9.1%; (4) yellow corn 90.9%, barley malt 9.1%. After conversion, the mashers were cooled and inoculated with 3% by volume of yeast from the regular plant yeast and set at 40 gal of mash per bushel of air-dry grain. The fermentations were incubated at 90°F usually for 72 hr. The alcohol yield in proof-gal per

<sup>1</sup> Present address: Hoffman-LaRoche, Inc., Nutley, New Jersey.

56-lb bushel of moisture-free grain was 5.2–5.5 for wheat, 5.6–6.0 for corn, 4.7–5.1 for rye, and 6.3–6.5 for granular wheat flour.

### Pilot Plant By-Product Recovery Equipment

A pilot plant recovery system of a suitable size was planned and put into operation to process the stillage from experimental 5-gal fermentations. The system involved the following equipment:

1. Metal screen with a drainage pan, constructed at a 15° angle, 36 inches long, 8 inches wide, with screen openings 1 mm in diameter on 0.075-inch centers.
2. Batch vacuum evaporators, 5- and 12-gal capacity, steam-heated.
3. Atmospheric double-drum dryer, maximum steam pressure 160 lb per sq inch; surface area of each drum 1 sq. ft.
4. Drying rack with vertical adjustment holding several infra-red drying bulbs.

### By-Product Recovery

The recovery procedure in the pilot plant operations was the same in all cases. A weighed quantity of beer (approximately 4 gal) was distilled under vacuum for one hr at a vapor temperature of 145–155°F (62.8–68.3°C) to remove the alcohol. Steam was then passed into the beer to bring it to a temperature range of 208–212°F (97.8–100.0°C). The hot stillage was adjusted to the weight of the original beer by adding water and was immediately screened. The rate of flow over the screen was controlled by use of a hand-operated wooden baffle. The grains (screenings) were manually pressed on the screen to remove most of the absorbed liquid. The weighed screen effluent was concentrated at 160–165°F (71.1–73.9°C) to a syrup of approximately 20% total solids. The syrup was converted to dried solubles on the drum dryer operating at 140–150 lb steam pressure, with a drum clearance of 0.015 inches and with a drum rotation rate of 2.75 rpm. As judged by appearance and odor, a satisfactory product was produced from all fermentations. The pressed distillers' grains were dried under infra-red lamps.

Corn stillage screened most easily, while granular wheat flour stillage screened very slowly. The other two were intermediate, with wheat stillage showing slightly greater screening ease than rye. The moisture-free total by-product yield per 56-lb bushel of air-dried grain was 18.5–20 lb for wheat, 16–17.5 lb for corn, 20–21.5 lb for rye, and 15–15.5 lb for granular wheat flour. The amounts of distillers' solubles and distillers' grains were about equal for wheat; the grains slightly exceeded the solubles for corn; the solubles slightly exceeded the grains for rye; and for granular wheat flour, the amount of solubles more than doubled the amount of distillers' grains. Definitions for the distillers' by-products have been given by the Association of American Feed Control Officials (1944).

In order to ascertain whether the pilot plant screening and pressure procedure would yield a product comparable to that obtained in commercial operations, samples of whole stillage were taken from plant operations and screened in the pilot plant equipment. These grains (screenings) are compared with the regular commercial products in Table I. The data show the pressed distillers' grains from the two operations to be quite comparable.

TABLE I  
ANALYSES OF PRESSED DISTILLERS' GRAINS PREPARED DURING PILOT  
PLANT AND COMMERCIAL OPERATIONS<sup>1</sup>

Trial	Process	Solids content	Proximate composition (dry-matter basis)			
			Protein	Fat	Fiber	Ash
		%	%	%	%	%
1	Pilot plant	23.9	31.1	9.2	12.9	2.1
	Commercial	26.2	31.4	10.1	13.8	1.9
2	Pilot plant	24.3	32.5	8.8	13.2	2.2
	Commercial	26.7	31.0	9.6	14.2	2.3
3	Pilot plant	25.5	30.9	11.4	13.0	2.1
	Commercial	26.7	31.0	11.3	13.1	2.0
4	Pilot plant	24.7	31.5	11.9	14.1	2.3
	Commercial	27.4	30.8	9.6	15.3	1.9

<sup>1</sup> The mash bill employed in the above study contained the following grains:

Corn	—28.13%
Wheat	—48.58%
Granular wheat flour	—10.56%
Barley malt	— 9.93%
Rye	— 2.80%

### Methods of Analysis

Moisture-free samples were obtained by drying at 105°C to constant weight. All protein data in the paper are nitrogen values multiplied by 6.25. Thiamine was determined by the thiochrome method of Hennessy (1941). Riboflavin was assayed by the method of Snell and Strong (1939). Samples were extracted with 0.1*N* HCl in an autoclave for 15 min at 15 lb pressure. Samples for the pantothenate assay were extracted with water at a pH of 6.5–6.8 for 15 min after which the microbiological method of Pennington, Snell, and Williams (1940) was followed. Crystalline d-calcium pantothenate served as the reference standard. Care was taken in both these assays to avoid the growth stimulants described by Bauernfeind, Sotier, and Boruff (1942). The Snell and Wright (1941) method for nicotinic acid was employed and the samples were extracted with 0.1*N* NaOH for 15 min in an autoclave.



### Experimental Results and Discussion

The proximate feed analyses and mineral composition of the by-products are presented in Tables II and III, respectively. As the cooking temperature of the grains was increased, a greater percentage of protein was found in the distillers' grains (screenings). Heat has

TABLE II  
CHEMICAL COMPOSITION OF ALCOHOL FERMENTATION  
BY-PRODUCTS FROM VARIOUS GRAINS

Fermentation by-product	Cooking temperature <sup>1</sup>		Proximate composition (dry-matter basis)			
			Protein	Fat	Ash	Fiber
	°C	°F	%	%	%	%
Wheat distillers' dried solubles	68.3	155	46.5	0.5	8.8	2.2
	100.0	212	39.0	0.8	10.1	2.5
	137.8	280	37.0	0.9	10.0	2.4
	155.6	312	35.8	0.6	10.1	2.3
Wheat distillers' dried grains	68.3	155	27.2	5.5	2.3	14.7
	100.0	212	37.9	4.7	2.8	12.0
	137.8	280	39.5	4.3	2.5	13.1
	155.6	312	42.6	—	2.1	13.9
Corn distillers' dried solubles	100.0	212	21.7	5.2	9.7	2.9
	137.8	280	28.5	6.6	9.5	3.8
	155.6	312	24.7	—	9.3	3.1
Corn distillers' dried grains	100.0	212	30.5	8.3	1.9	12.1
	137.8	280	32.3	10.3	2.0	12.8
	155.6	312	33.9	11.4	1.8	13.2
Rye distillers' dried solubles	68.3	155	40.4	0.7	8.0	2.6
	137.8	280	36.4	0.7	8.5	2.8
Rye distillers' dried grains	68.3	155	24.0	6.3	2.2	13.0
	137.8	280	28.9	6.8	2.3	13.4
Granular wheat flour distillers' dried solubles	68.3	155	46.0	1.1	5.3	2.2
	137.8	280	42.8	1.3	4.9	2.9
Granular wheat flour distillers' dried grains	68.3	155	27.0	4.5	2.6	15.7
	137.8	280	44.0	3.2	2.4	11.2

<sup>1</sup> Refers to maximum temperature reached in the cooking cycle.

long been recognized as a method of denaturing proteins and this makes them more insoluble. Once the protein is precipitated it is more easily retained among the other coarser grain residues as they pass over the screen. Conversion of the starch to alcohol and carbon dioxide results in a concentration of the remaining nutrients of the original grain in the composite by-products of fermentation. Conversely then, from the data on the composition of the by-products and with the use of

quantitative by-product recovery data, the analyses of the original cereal mixture grain can be computed as a check on the by-product analyses. For example, when this is carried out on the wheat by-products the computations show the following composition for the wheat mixture (92% wheat, 8% barley malt) on an air-dry basis: protein 13.1%, fat 1.9%, fiber 2.7%, ash 2.1%, calcium 0.12%, and

TABLE III  
MINERAL COMPOSITION OF ALCOHOL FERMENTATION  
BY-PRODUCTS FROM VARIOUS GRAINS

Fermentation by-product	Cooking temperature <sup>1</sup>		Calcium <sup>2</sup>	Phosphorus <sup>2</sup>
	°C	°F	%	%
Wheat distillers' dried solubles	137.8	280	0.52	1.70
Wheat distillers' dried grains	137.8	280	0.22	0.50
Corn distillers' dried solubles	155.6	312	0.51	1.50
Corn distillers' dried grains	155.6	312	0.15	0.24
Rye distillers' dried solubles	137.8	280	0.46	1.48
Rye distillers' dried grains	137.8	280	0.13	0.37
Granular wheat flour distillers' dried solubles	137.8	280	0.48	0.91
Granular wheat flour distillers' dried grains	137.8	280	0.20	0.35

<sup>1</sup> Refers to maximum temperature reached in the cooking cycle.

<sup>2</sup> Dry-matter basis.

phosphorus 0.38%. Computations from the rye by-products show the following composition for the rye mixture (90.9% rye, 9.1% barley malt) on an air-dry basis: protein 12.0%, fat 1.3%, fiber 2.7%, ash 2.0%, calcium 0.11%, and phosphorus 0.38%. In both cases it will be noted that the analyses are in fairly good agreement with accepted values for the mixtures of cereal grains, with the exception of the calcium content. Usually these grains contain 0.04–0.06% calcium. The difference between these values and the 0.11–0.12% obtained from the above computations is attributed to the calcium supplied by the use of limestone-bearing well water in the process. Boruff, Smith, and Walker (1943) reported the calcium content of limestone-bearing well water to be 125.7 ppm. About 33 to 38 gal of water are used per bu of grain fermented, supplying the additional 0.06–0.07% calcium.

Thiamine, riboflavin, nicotinic acid, and pantothenate values for the various by-products are reported in Tables IV and V. In the case of the corn and wheat distillers' dried solubles, a higher thiamine content was observed when they were prepared from grain mashs cooked at 212°F than at 312°F, which indicates that greater destruction of thiamine was caused by the higher cooking temperature. The riboflavin,

which was a variable in this report, is the cooking of the grain. While the maximum cooking temperature was maintained for only 15 min, the entire heating period for the mash to reach the maximum temperature and to cool to and below the malting temperature, required about 1.5 hr. During this time the pH was 5.5–6.2. The results in Table IV show that more thiamine was destroyed upon cooking to a temperature of 312°F (155.6°C) than to 212°F (100°C).

In the evaporation of the screen effluent during the pilot plant study, a temperature of 160–165°F (71.1–73.9°C) was applied for approximately 6 hr. The pH of the screen effluent was 3.8–4.1. In the commercial process, only 4 to 5 hr are required, and the temperature varies from 220°F (104.4°C) in the first evaporator to 130°F (54.4°C) in the finishing pan. In the drum-drying of the concentrated syrup the product is subjected to a high temperature for a short period of time, about 20 sec, and usually there is little or no destruction of pantothenate in this time. If the knife blades are raised on the drum and the product permitted to remain there for 100 sec, about 50% of the pantothenate present in the syrup is destroyed. The available evidence at the moment shows no loss of riboflavin or nicotinic acid during the recovery of the by-products.

Beadle, Greenwood, and Kraybill (1943), in studying the stability of thiamine to heat, reported that it was a function not only of pH but also of the electrolyte system. At a pH of 5.4 and at boiling temperature for 1 hr, 57% of the thiamine in pure water was destroyed, while only 10% was destroyed in a dilute acetate buffer. Destruction was greater at pH values higher than 5.4. Frost (1943) found the rate of destruction of pantothenate to be a function of pH and temperature and also to be affected by other substances in aqueous solution. He found that if a 1% dextrorotatory calcium pantothenate aqueous solution at pH 4.0 was stored at 60°C for 5 days that 26% of the activity was destroyed, while in a dilute phosphate buffer solution 10% was destroyed.

Synthesis of riboflavin by microorganisms, bacteria, yeast, and molds has been reported by a number of investigators. Hence riboflavin synthesis does not seem unlikely here. Laufer, Davis, and Saletan (1942) assayed the raw materials, the intermediate, and the end products of both an ale and a lager brew for vitamin content to determine their fate in the brewing process. On the basis of the data for the products assayed, the authors concluded that there is some loss of thiamine in the brewing process due to destruction of the vitamin, that there is relatively little loss or gain of nicotinic acid, and that a possible synthesis of pantothenate and riboflavin by yeast is indicated.

### Summary

The proximate, mineral, and vitamin compositions of fermentation by-products in the production of alcohol from corn, wheat, granular wheat flour, and rye have been presented. As the cooking temperature of the cereal grains was increased, a greater percentage of the protein in the total by-products was found in distillers' grains (screenings). The use of limestone-bearing water in the process significantly increased the calcium content of the by-products. In the production of the fermentation by-products, some thiamine and pantothenate were destroyed, presumably by heat treatment at sensitive pH levels. The nicotinic acid content of the by-products was due primarily to a concentration of the nicotinic acid present in the original grains, while the riboflavin content of the by-product was derived both from the riboflavin in the original grains and from synthesis by microorganisms.

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## PRODUCTION OF STARCH FROM WHEAT AND OTHER CEREAL FLOURS

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Normally about 98% of the starch production in the United States (approximately 2,500 million pounds annually) is accounted for by the wet milling of corn. Since 1940 starch production has increased, the wet-milling industry having processed 130 million bushels of corn in 1942 compared with about 75 million bushels annually prior to 1940. The recent shortage of corn on the cash market, coupled with the increased demand for starch and its conversion products for use in food and essential industries, has directed attention toward the use of other cereal grains for starch production. Wheat, in particular, has received consideration as an alternative source of starch, the fermentation industry having turned to it in 1942 as a partial replacement for corn in alcohol production. The utilization of wheat and other cereal grains as raw material for maintaining or increasing starch production should be based on processing methods which permit: (a) production of starch of low protein content in good yields for use as such or for fermentation or conversion to glucose sirup or sugars, (b) recovery of by-product protein, (c) adaptation to existing plant facilities with a minimum of new installations involving critical materials, and (d) use of different varieties of wheat and other cereal grains if required.

Wheat is the earliest recorded material from which starch was prepared. The technical and patent literature on starch production, however, is largely confined to the use of corn for wet-milling operations. In some cases applicability of the steps to wheat and other grains is claimed, although there is little or no evidence of actual practice on these grains. The two well-known processes for the production of starch from wheat grain are the Halle or fermentative process and the Alsatian or nonfermentative process. In these processes a water steep with subsequent wet milling is employed. Recent studies by Slotter and Langford (1944) have demonstrated the applicability of modern corn wet-milling practice to wheat grain. Such a process would be suited to the utilization of wheat in existing wet-milling plants when desired.

Existing wheat starch manufacturers have generally found it advantageous to use flour as the raw material. Wheat flour has a

<sup>1</sup> This is one of four regional research laboratories operated by the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

higher starch content than the grain, can be processed more rapidly with less plant space and equipment, and its use minimizes by-product recovery, since most of the bran and fiber is retained at the flour mill. The main disadvantage of the use of flour is its higher cost on an equivalent starch basis.

The most generally used method, at present, for producing starch from wheat flour is the Martin process (Eynon and Lane, 1928), based on washing the starch out of a dough. This process provides ready recovery of by-product wheat gluten and a good yield of starch. The Martin process, however, is not readily adaptable to large-scale operation, is limited in applicability to wheat flour having good doughing properties, and cannot be used to separate starch from other cereal flours. Modifications of the Martin process have been suggested (Eynon and Lane, 1928) which eliminate the dough-washing step by substitution of a centrifugal separation of the gluten and starch from a dough or paste. These methods, however, do not effect a clean-cut separation of starch and protein.

In addition to these primarily mechanical procedures for wheat starch production, methods largely dependent on chemical treatment, particularly with alkali, have been proposed. Alkali disperses or dissolves the protein, thereby facilitating separation of the starch in a state of high purity. Jones (1841) patented a process for the separation of starch from rice or wheat meal by an alkali treatment which is still used as the basis for rice starch production. However, no detailed information is available in the literature on the use of alkali in the production of starch from wheat, wheat flour, or other cereal grains except rice and corn industrially.

This paper deals with an investigation of processing conditions and methods which might be used for preparing starch and protein from flour by alkali treatment. Some information is available in the literature on the action of alkali on wheat protein and wheat flour. Relative to the problem of starch production these data are incomplete. The present studies therefore may be resolved into two sections: (a) establishment of background information on the behavior of the starch and protein in wheat flour in the presence of alkali, and (b) application of this knowledge to the preparation of starch and by-product protein.

### Experimental Methods and Results

*Flours Used.* Most of the flour samples from wheat and other cereal grains used for these studies were prepared in an experimental Buhler flour mill using 10XX bolting silk (110 mesh). With Rex soft white wheat and Fulton oats the tendency of the flour to "ball-up" on the fine silk necessitated the use of a No. 48 or No. 64 grit gauze

(50 to 60 mesh) as the finest bolting cloth in the system. The samples of granular and second clear wheat flour and white rye flour were commercially prepared products. The starch and protein content and the milling yield of the various flour samples used are shown in Table I.

TABLE I  
ANALYSES OF FLOUR SAMPLES  
(All data on moisture-free basis)

Kind of flour	Milling yield	Starch content	Protein content
	%	%	%
No. 2 Dark Northern Spring wheat (from Commodity Credit Corporation)	70	78	13.7
Thatcher hard red spring wheat	72	76	16.4
No. 2 Hard Winter wheat (from Com- modity Credit Corporation)	70	80	12.7
American Banner soft white wheat	70	81	10.6
Rex soft white wheat	74	81	7.0
Second clear (hard red spring wheat) <sup>1</sup>	3	64	20.9
Granular wheat (grits) <sup>1</sup>	60	77	12.9
White rye <sup>1</sup>	55	76	9.6
Trebi barley	45	79	8.4
Fulton oats	62	72	13.0
Illinois Hybrid No. 972 corn	21	79	7.5
Pink kafir (sorghum)	33	88	6.9
Colusa rice	59	92	4.7

<sup>1</sup> Samples commercially prepared.

The protein value, based on Kjeldahl nitrogen, was calculated as  $N \times 5.7$  for all except corn and sorghum, for which the factor 6.25 was used. The starch was determined polarimetrically by a modification (Clendenning, K. A., private communication) of the Hopkins' procedure (1934).

*Dispersing Action of Alkalies on Wheat Protein.* The dispersing action of aqueous alkalies on wheat protein was determined by a modification of the procedure for the determination of water-soluble protein nitrogen according to A.O.A.C. (1940). Twenty g of flour was mixed with 200 ml of the alkali solution and shaken frequently for 30 min. The mixture was then allowed to settle for 2 hr and the nitrogen content of the supernatant was determined. The apparent protein solubility, expressed in percent, was calculated as follows:

$$\text{Apparent protein solubility} = \frac{\text{mg } N \text{ per ml supernatant} \times 200}{\text{mg } N \text{ per g flour} \times 20} \times 100.$$

This represents the percent of the total protein which is dispersed, under the conditions used, to such a degree that it does not readily settle by gravity.

Measurements were made of the protein-dispersing power of solutions of sodium, potassium, calcium, and barium hydroxide and of sodium carbonate, using American Banner soft white wheat flour. The observed apparent protein solubilities, given in Table II, indicate

TABLE II  
DISPERSION OF PROTEIN IN AMERICAN BANNER WHEAT  
FLOUR BY VARIOUS ALKALIES

Alkali		Apparent solubility	pH of mixture <sup>1</sup>
		%	
NaOH	0.03 <i>N</i>	101	11.8
KOH	0.03 <i>N</i>	100	11.7
NaOH	0.01 <i>N</i>	95	10.9
KOH	0.01 <i>N</i>	98	10.8
Ca(OH) <sub>2</sub>	0.05 <i>N</i>	92	11.7
Ba(OH) <sub>2</sub>	0.05 <i>N</i>	90	11.7
Na <sub>2</sub> CO <sub>3</sub>	0.3%	66	10.3

<sup>1</sup> Measured with glass electrode, uncorrected for alkali salt errors.

that maximum removal of protein from wheat flour is effected by sodium or potassium hydroxide. If a higher protein content in the starch can be tolerated, the use of calcium hydroxide offers the advantage of low raw material cost. Sodium hydroxide was used for most of the studies dealing with the effect of various factors on the production of starch with a low protein content.

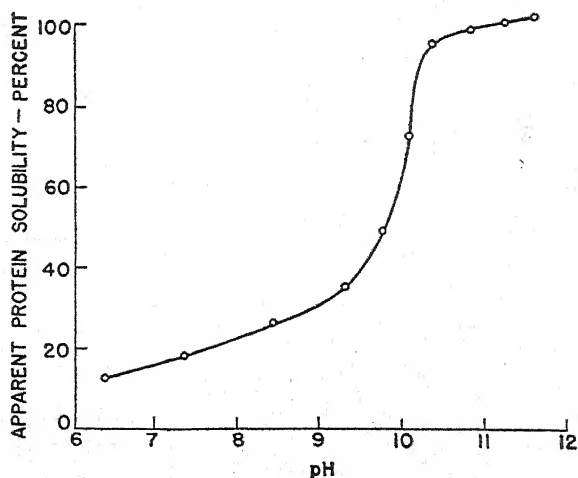


Fig. 1. Relationship of the extent of wheat protein dispersion to the pH of the mixture of flour and aqueous sodium hydroxide.

The relationship between the apparent protein solubility and the pH of the mixture of aqueous sodium hydroxide and No. 2 Hard Winter wheat flour (12.6% protein, moisture-free basis) is shown in Figure 1.



For the flour from other varieties of wheat and from rye and barley it was found that the use of 0.01*N* NaOH resulted in a pH of 10.4 to 10.8 and an apparent protein solubility of about 95%, while 0.03*N* NaOH gave a pH of about 11.4 to 11.7 and essentially complete protein dispersion. For any given flour the pH of the mixture appeared to be primarily dependent on the ratio of flour to sodium hydroxide and influenced only to a minor extent by variations of the amount of water within practicable limits. The results show that, for maximum dispersion of the flour protein, the mixture of flour and sodium hydroxide should have a pH above 10.5.

The dispersion of the protein in the flour was very rapid in sodium hydroxide solutions. The shortest period for which a measurement was made was 10 min, in which time dispersion was complete. In

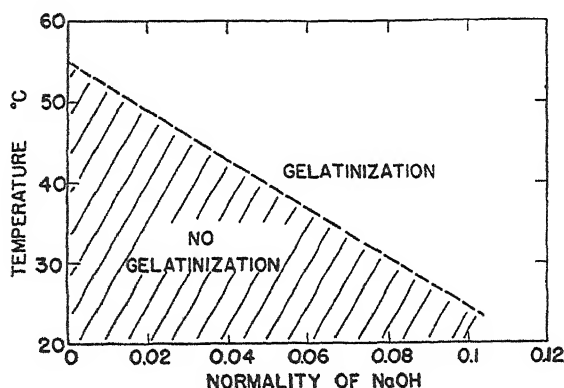


Fig. 2. Relationship of temperature and amount of sodium hydroxide to gelatinization of starch in wheat flour; 10 g flour per 100 ml of sodium hydroxide; 4-hr treatment.

pilot-plant operations, for which highly efficient mixing equipment was not available, periods of 15 to 30 min were usually allowed for the dispersion step.

*Starch Gelatinization.* Approximate working limits of temperature and sodium hydroxide concentration for the alkali treatment of flour without gelatinizing the starch were established in the following manner. Ten-g samples of American Banner soft white wheat flour were mixed with 100-ml portions of 0.1*N*, 0.05*N*, and 0.03*N* NaOH at about 25°, 30°, 40°, and 50°C and allowed to stand for 4 hr. Gelatinization was followed microscopically, using as an index the loss of birefringence and the staining by benzopurpurin of the granules. The approximate range of temperature and strength of sodium hydroxide in which little or no gelatinization of the starch occurred is shown by the shaded area in Figure 2. The pH was about 11.8 for

a mixture of the flour with 0.03*N* sodium hydroxide at room temperature.

*Protein Recovery.* Recovery of most of the protein was effected by acidifying the starch-free alkaline solution. The optimum pH for precipitating the protein was determined as follows:

An alkaline wheat protein dispersion was prepared by centrifuging the alkali-insoluble solids from a mixture of 100 parts (moisture-free basis) No. 2 Hard Winter wheat flour, 1,250 parts water and 1.5 parts NaOH (solid). Aliquots of this solution were adjusted to various pH values with H<sub>2</sub>SO<sub>4</sub>; the precipitated protein was separated by centrifuging; and the protein contents of the supernatants determined. The relation between pH and the percentage of protein precipitated is shown in Figure 3. Essentially the same results were obtained when

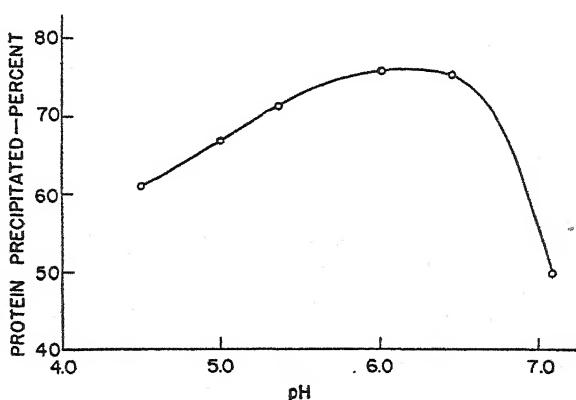


Fig. 3. Effect of pH on the extent of precipitation of wheat protein by acidification of the starch-free alkaline dispersion with sulfuric acid.

other acids, such as HCl, SO<sub>2</sub>, and CO<sub>2</sub>, were used, the lowest pH readily obtained with the last being about 6.3.

The flocculation and settling behavior of the protein was variable. At pH 6.0 and higher, frequent failures to flocculate and settle were encountered. At pH 5.5, however, flocculation occurred regularly with all of the wheat flours studied in this region of flour concentration. This has led to a preference for pH 5.5 for protein recovery in the procedures for processing flour despite the higher percentage of protein precipitated in the region of pH 6.0.

In the pH range of 5.0 to 6.0 the flocculated protein settled by gravity at a rate of between 1 and 2.5 ft per hr to occupy one half to one third of the total volume of fluid. Centrifugation at about 800 times gravity gave a soft protein cake containing 70 to 80% moisture. When dried, this cake became hard and brittle and had a protein

content of about 85% ( $N \times 5.7$ ), moisture-free basis, representing from 67 to 77% of the protein originally present in the alkaline solution.

The protein precipitated by acidification was finely divided and did not filter readily. Evidence was obtained which confirmed the findings of the Overly Biochemical Research Foundation, Inc. (private communication, 1943), that the use of a lignin sulfonate as a precipitation aid might be effective in giving recovery of protein in a filterable form. The proportion of dry lignin sulfonate required, however, was undesirably high, being approximately equal in weight to the protein to be precipitated.

The concentration of protein in the alkaline solution was increased either by raising the flour concentration or, more practicably, by using the alkaline starch-free liquor from one flour dispersion for the treatment of additional portions of flour (with the necessary addition of alkali) before precipitating the protein. A concentration of protein and other alkali solubles was reached, however, at which the protein failed to flocculate and settle, even at pH 5.5. In qualitative experiments on wheat flour, such failure was encountered with an alkaline solution equivalent to that from the treatment of 1 part flour with 3 parts aqueous alkali.

The wheat protein recovered by precipitation from the alkaline dispersion had undergone some chemical change. When the solutions were acidified, the odor of hydrogen sulfide was noted, probably indicating changes involving cysteine. The recovered protein was no longer glutinous in character, although its solubility in solvents such as acetic acid or alcohol was not greatly different from that of "native" wheat gluten.

*Processes for Starch Separation after Alkali Treatment of Flours.* Two processes, centrifuging and tabling, were used for separating starch from flour after alkali treatment. The details of conditions and processing steps, based on the preceding data and on trial processing, were chosen with the objective of providing applicability to a wide variety of flours. To facilitate the separation of a maximum of protein from the starch, a pH above 10.6 was used, thus insuring essentially complete dispersion of the protein by sodium hydroxide (see Fig. 1). An upper limit of pH 11.8, obtained with about 1.5 parts NaOH per 100 parts flour, was set, along with an upper temperature limit of 35°C, to avoid the region of starch gelatinization shown in Figure 2. Exploratory experiments showed that increasing the flour concentration, although resulting in an economy in the volume of fluids, decreased the rate of settling of the starch and increased the amount of protein in the liquor held in the starch layer. For the tabling of starch from the alkaline wheat flour suspension, a concentra-

tion of 1 part flour in 12.5 parts of 0.03*N* NaOH was found most practical, while for centrifuging, 1 part flour in 6 parts of 0.03*N* NaOH could be used for most flours.

The two processes for starch and by-product protein production, as described below in detail, illustrate the use of representative conditions and processing steps. Considerable variation of the processes is possible depending on the ingenuity of the processor, the equipment used, and the purity required of the products. The results of the processing of different flours are given to indicate the applicability to a wide variety of raw materials.

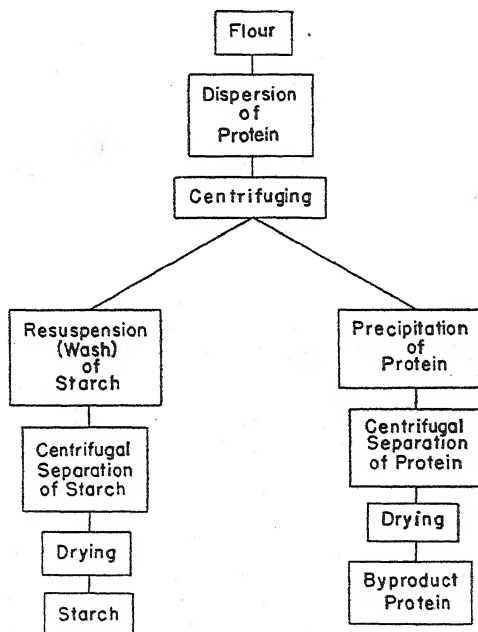


Fig. 4. Centrifuging process flow sheet for obtaining the products in Table III.

*Centrifuging Process and Application to Various Wheat Flours.* In the centrifuging process the starch is separated in a single fraction, together with the small amounts of other alkali-insoluble solids, for the production of a low-protein crude starch by a minimum number of operations. The general procedure adopted for the centrifugal separation of starch from alkali-treated flour is outlined in the flow sheet (Fig. 4).

For the preparation of starch, 150 g (100 parts) of flour (moisture-free basis) was mixed with 900 ml of 0.03*N* NaOH (600 parts aqueous solution containing 0.75 parts NaOH) at between 25° and 35°C with

rapid stirring until all lumps of flour had disintegrated, usually within 15 min. This flour suspension (about 8.0° Baumé) was introduced into an imperforate basket centrifuge at such a rate that the liquor overflowing the rim of the basket was starch-free. The cake of crude starch was resuspended in about 800 ml of water with vigorous stirring, and the suspension introduced into the centrifuge basket as before, without neutralization. The resulting starch was dried at 40°C in a mechanical convection oven. When a neutralized starch was desired, the washed starch was resuspended and the pH adjusted.

TABLE III

YIELD OF STARCH AND PROTEIN FROM VARIOUS FLOURS—CENTRIFUGING PROCESS  
(All data calculated on moisture-free basis)

Kind of flour	Crude starch	Washed starch <sup>1</sup>			Precipitated protein		
	Protein content	Yield	Starch recovery	Protein content	Yield	Protein content	Protein recovery
	%	Lb/100 lb flour	%	%	Lb/100 lb flour	%	%
Rex soft white wheat	0.9	86	101	0.3	6	70	63
No. 2 Hard Winter wheat	1.7	83	101	0.4	10	87	71
No. 2 Dark Northern Spring wheat <sup>2</sup>	2.7	82	100	0.4	11	85	65
Thatcher hard red spring wheat <sup>2</sup>	2.5	79	101	0.4	13	88	69
Second clear (hard red spring wheat) <sup>2</sup>	2.7	71	105	0.6	20	82	78
Granular wheat	3.1	81	98	1.1	9	87	62
White rye	1.5	80	101	0.4	3		
Trebi barley	1.0	83	99	0.5	8	72	67
Fulton oats	2.0	74	98	0.6	19	57	81
Illinois Hybrid No. 972 corn	2.5	85	100	1.5	9	54	61
Pink kafir (sorghum)	4.1	92	98	3.4	4	59	30
Colusa rice	1.8	93	97	1.0	4	69	57

<sup>1</sup> The nitrogen content of the starch is expressed, for uniformity, as protein, calculated as  $N \times 6.25$  for corn and sorghum and  $N \times 5.7$  for the other starches, although the nitrogenous material associated with the starch granules is quite different from the bulk of the flour protein. About 0.02 to 0.04% N (0.1 to 0.3% protein as calculated above) is apparently either incorporated in the starch granules or firmly adsorbed by them, since this is the lower limit of nitrogen in samples of cereal starches prepared by various methods.

<sup>2</sup> Starch centrifuged from suspension of 1 part flour in 6 parts 0.06N NaOH solution. For all other flours 0.03N NaOH solution was used in the same proportions.

<sup>3</sup> Did not precipitate at the flour concentration used.

By-product protein was recovered from the alkaline liquor from the first centrifugation by acidifying to pH 5.5 with dilute sulfuric acid. The precipitated protein was collected by centrifuging and dried. No attempt was made to recover the protein from the starch wash water, which in continuous processing would be used for treatment of the next batch of flour. This wash water usually contained about 10–15% of the total flour protein and would probably augment the recovery of precipitated protein by at least half this amount on recycling.

The results of the application of this process to wheat flour of different grades and from representative varieties of wheat are shown in Table III. The protein content of the crude starch is included as an indication of the quality of starch which would be obtained without any washing.

After one washing, the starch from the straight flours had a protein content of 0.3 to 0.6%, with the exception of that from the No. 2 Dark Northern Spring and the Thatcher hard red spring wheat flours, which contained 1.2% and 0.9% protein, respectively. From these flours, starch with only 0.4% protein was easily prepared by using either an additional 600 parts water (1,200 parts of 0.015*N* NaOH solution) or double the strength of sodium hydroxide (600 parts of 0.06*N* NaOH solution) for the initial flour dispersion. The higher protein content of the starch from the second clear and the granular wheat flour may be attributed to the presence of larger particles of bran or fiber from which the nitrogenous material is not readily extracted.

Starch obtained by this process contains all the alkali-insoluble solids of the flour which lower the starch content to 94 to 98% as compared with a purity of about 99% for the highest-quality starch prepared by the usual commercial methods. The main nonprotein impurities consist of the cellulosic cell-wall fragments and bran. This material, along with the gelatinized starch arising from granules damaged by milling, contributes gumminess or stickiness to the centrifuge cake and prevents ready filtration of the starch. The starch can be separated by centrifugation into prime-quality and lower-quality starch, as is done in commercial rice starch processing (Eynon and Lane, 1928), by taking advantage of the difference in rate of settling of the two fractions.

*Tabling Process and its Application to Various Wheat Flours.* In the tabling process the starch is recovered in two fractions: (a) the prime-quality starch which deposits on the table, and (b) the "tailings starch" obtained by centrifugation of that part of the suspension passing over the end of the table. An outline of the procedure used for the separation of starch by tabling the alkaline flour suspension is given in the process flow sheet (Fig. 5).

For the preparation of starch, 454 g (100 parts) of flour (moisture-free basis) was added to 5.7 l of 0.03*N* NaOH (1,250 parts aqueous solution containing 1.5 parts NaOH) at 25° to 35°C with rapid stirring. The suspension (about 4.1° Baumé) was run onto a starch table at a rate of about 170 ml per min, the table being 3 inches × 12 ft in size with a pitch of one fourth inch in 10 ft. The tailings were run directly into an impermeate basket centrifuge. One l of water was then run

onto the table at a rate of about 330 ml per min to displace the supernatant layer of alkaline liquor and to sweep away loose solids overlying the starch cake on the table. The starch was removed from the table and the starch milk was adjusted to pH 5.5–6.0 with dilute  $H_2SO_4$  and screened through No. 17 standard bolting silk. The starch milk, made up to a volume of about 3 l (approximately 6.2° Baumé), was

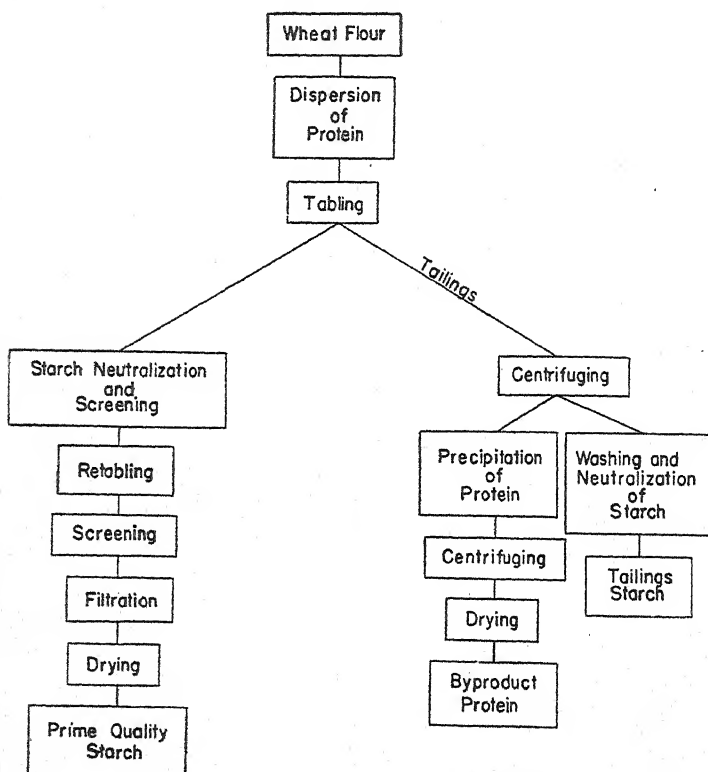


Fig. 5. Tabling process flow sheet for obtaining the products in Table IV.

tabled at a rate of about 330 ml per min. The tabled starch was re-suspended with about 800 ml of water, screened through silk as before, filtered by suction on a Büchner funnel, and dried.

The solids from the centrifugation of the first tabling tailings were washed twice by suspension in about 1 l of water and centrifuging, the second washing being accompanied by adjustment to about pH 6.0 with sulfuric acid. Precipitable protein was recovered from the alkaline liquor from the initial centrifuging of the first tailings in the same manner as in the centrifuging process.

The application of the tabling process to flour from different varieties of wheat gave the products shown in Table IV. For brevity, the starch obtained from the second tabling tailings is not included in the tabulation, but usually amounted to about 2-6% of the total starch. The prime-quality table starch constituted between 68 and 83% of the starch originally present in the flour, most of the recoveries

TABLE IV

YIELD OF STARCH AND PROTEIN FROM VARIOUS FLOURS—TABLING PROCESS

(All data calculated on moisture-free basis)

Kind of flour	Tabled starch			First tailings starch			Precipitated protein		
	Yield	Starch recovery	Protein content	Yield	Starch recovery	Protein content	Yield	Protein content	Protein recovery
	Lb/100 lb flour	%	%	Lb/100 lb flour	%	%	Lb/100 lb flour	%	%
<i>Laboratory runs</i>									
American Banner soft white wheat	67	83	0.2	14	16	0.5	8	77	57
Rex soft white wheat	59	73	0.2	18	19	0.5	4	70	39
No. 2 Hard Winter wheat	57	72	0.3	16	19	0.4	9	84	57
No. 2 Dark Northern Spring wheat	56	71	0.3	19	22	0.4	8	79	46
Thatcher hard red spring wheat	55	72	0.3	18	22	0.5	11	90	62
Second clear (hard red spring wheat)	44	68	0.2	21	26	0.7	16	84	65
White rye <sup>1</sup>	55	72	0.1	20	24	0.6	8	48	42
Trebi barley <sup>1</sup>	50	63	0.2	26	31	0.5	9	68	69
Illinois Hybrid No. 972 corn	67	85	0.5	6	6	2.5	8	56	61
Pink kafir (sorghum)	69	80	0.9	7	7	8.9	5	57	41
<i>Pilot-plant runs</i>									
No. 2 Hard Winter wheat	59	74	0.2	17	20	0.5	9	79	56
White rye <sup>1</sup>	55	73	0.1	22	24	1.2 <sup>2</sup>	—	—	—
Trebi barley <sup>1</sup>	47	60	0.2	31	33	0.7	—	—	—

<sup>1</sup> Starch tabled from a suspension of 1 part flour in 25 parts 0.015N NaOH solution. All other tablings were from a suspension of 1 part flour in 12.5 parts 0.03N NaOH.

<sup>2</sup> Not washed.

being about 72%. The protein content of this starch was 0.2 to 0.3%, which is comparable to that of the highest quality starch prepared by the usual commercial methods. The first tailings starch fraction, which accounts for 16 to 26% of the starch in the flour, contained 0.4 to 0.7% protein and had a starch content of 88 to 94% when obtained from the straight flours. The first tailings starch fraction from the second clear flour contained only 78% starch as a result of the higher bran and fiber content of this flour, most of this material being recovered in the tailings. The protein recoveries by precipitation were from 46 to 65%, being lower than in corresponding runs by the centrifuging process because of the lower concentration of protein in



the tabling process. The purity of the recovered protein was between 70 and 90%.

The yields of products in the different fractions are representative and were usually reproducible, under fixed conditions, within about 2%. The quantities obtained from a given flour are dependent on the rate of tabling, the concentration of flour and sodium hydroxide, the pitch and relative size of the starch table, and other details of manipulation.

Recycling of process liquors is advantageous in general practice. The alkaline liquor from the tailings of the first tabling can be used, with the addition of alkali, for the treatment of at least one further batch of flour before precipitating the protein. If a still higher concentration of alkali solubles is built up by several such recyclings the protein fails to precipitate when the alkaline solution is acidified. The entire tailings from the retabling of the starch can be used for the primary treatment of another batch of flour, care being taken while adding the necessary alkali that the starch in the tailings is not gelatinized by local regions of high alkalinity.

*Composition and Properties of Tailings Starch.* A typical partial chemical analysis of a washed and neutralized wheat tailings starch, expressed on the moisture-free basis, is given below:

	Percent
Starch	= 93.8 (polarimetric)
Protein	= 0.44 ( $N \times 5.7$ )
Pentosans	= 1.04
Fatty acids	= 0.65
Ash	= 0.60
Undetermined	= 3.5

Pentosans were determined by the official A.O.A.C. (1940) procedure, the distillate being redistilled and the furfural precipitated by thio-barbituric acid. Fatty acids were determined by acid hydrolysis and extraction from the hydrolysate. The ash content was determined by incinerating the starch at 700°C for 3 hr.

The material not accounted for in the above analysis probably is largely cellulosic in nature.

This tailings starch from the first tabling contains the lighter and often more voluminous alkali-insoluble solids of the flour which fail to deposit on the table. The protein content is very low, in contrast to the tailings or "amyloextrin" fraction obtained in starch production by the dough-washing procedure (MacMasters and Hilbert, 1944a) or the wet milling of wheat (Slotter and Langford, 1944). Microscopic examination of this material showed that it is made up of very small starch granules, gelatinized and swollen starch granules, and cell-wall fragments. This combination of solids offers technical

difficulties in processing. Filtration is slow, if not impossible. Centrifugation in an imperforate basket gives a very soft and gummy cake. When the product is dried in a mechanical convection oven at 40°C, a hard, horny mass is obtained, although vacuum drying at room temperature gives a fairly friable product. Therefore, it is desirable to use the tailings starch while still wet, *e.g.*, for hydrolytic conversion or fermentation, and with as little additional processing as possible for purification.

*Pilot-Plant Preparation of Wheat Starch by Tabling.* The tabling process used for the pilot-plant preparation of wheat starch was the same as that used in the laboratory. Fifty lb of flour (moisture-free basis) was mixed with 75 gal of water containing 0.75 lb of NaOH. The flour suspension was run onto a starch table, 1 ft × 40 ft in size with a pitch of about 0.5 inch in 10 ft, at a rate of about 0.6 gal per min (0.9 gal/sq ft/hr). The results given in Table IV are in agreement with those for the laboratory processing of the same flour. No difficulties were experienced in this larger-scale application of the procedure.

*Applicability to Flour from other Cereal Grains.* The centrifuging and tabling processes for recovering starch after alkali treatment were applied to rye, barley, oat, rice, corn, and sorghum flours without modification except in the amount of water used in the initial dispersion of rye and barley flours for tabling. Since these studies were designed to show the effectiveness of the wheat flour processing conditions for other flours, no attempt was made to establish optimum conditions for the individual flours. The yields of products obtained from these flours are shown in Tables III and IV.

The centrifuging process used with rye, barley, and oat flours gave starch comparable in purity to that from wheat flour, the protein content being 0.4 to 0.6%. The starch from corn, sorghum, and rice flours had a higher protein content, 1.0 to 3.4%, due to insolubility of a fraction of the protein in the alkali and/or mechanical retention of protein in vitreous particles of flour which are not broken up during the short treatment with alkali. The protein recovered by precipitation was between 57 and 81% of the flour protein for all but sorghum and rye flours. The low recovery of protein from sorghum flour was due to incomplete dispersion of the protein, since 51% of the flour protein was recovered in the starch cake. The alkaline liquor from the rye flour did not give a precipitate of protein on acidification when the prescribed flour concentration was used. The use of a more dilute dispersion mixture, containing 1 part rye flour in 12 parts 0.03*N* NaOH solution, permitted fairly satisfactory precipitation and recovery of about 62% of the protein in the rye flour. Oat and corn flours ap-

peared to have a higher alkali-binding capacity, since the addition of more alkali was necessary to bring the pH of the mixture above 10.6.

The tabling process, as described for wheat flour, was less applicable than the centrifuging process to other cereal flours. Rice and oat flours could not be processed by tabling because the starch, being composed of very small granules, settled too slowly to permit retention on the table at the rates of flow used. The alkaline protein dispersion from rye and barley flours was rather slimy and viscous, so that a more dilute flour suspension (containing twice the quantity of water used for wheat flour) was necessary to provide satisfactory initial deposition of starch on the table. The retabling of the starch, however, proceeded as described for wheat starch to yield prime-quality starch having the very low protein contents of 0.1 and 0.2%, and representing starch recoveries of 72 and 63%, from rye flour and barley flour, respectively. Corn and sorghum flours gave higher recoveries of starch on the table, but the protein contents were 0.5 and 0.9%, respectively.

The pilot-plant processing of barley and rye flours, using 50 lb of flour in each case and twice the quantity of water used for wheat flour, proved entirely comparable to the laboratory processing, as shown by the results presented in Table IV. Protein recovery was not studied in these runs.

### Evaluation of the Alkali Process for Starch Production

The applicability of the alkali process to a wide variety of raw materials permits a choice on the basis of availability and economy. The general success in the preparation of starch from all types of wheat flour studied indicates the feasibility of using such materials as low-grade wheat flour, flour of limited value for baking, such as that from some of the soft white wheats of the Pacific Northwest, and probably flour from damaged grain which is of such quality that it is unsuitable for food. That the starch from damaged wheat is generally of normal quality has been shown by MacMasters and Hilbert (1944). While wheat flour appears to be the most suitable raw material, rye, barley, and oat flours can also be processed with little or no modification of the steps. Less satisfactory results were obtained with corn, sorghum, and rice flours, the separation of protein from the starch not being as complete under the conditions used.

Starch which is well suited for use as such, for conversion to glucose or malt sirups, or for fermentation can be prepared by the alkali process. The quality of the starch is governed by the separation method used and the extent of purification. The tabling process for starch separation and fractionation is adapted to the production of a major fraction of very high-quality starch free of fiber and cell-wall

fragments and having a protein content comparable to that of the best commercial starches. This prime-starch fraction, which is approximately 70 to 80% of the starch in the flour, can be filtered and dried for use as such or converted into other products. The tailings starch is of lower quality, containing fiber, cell-wall fragments, and gelatinized and small starch granules, but relatively little protein. It would preferably be converted to lower quality sirups or used for other purposes requiring wet starch of fair purity.

The centrifuging process is advantageous for the preparation of starch that is to be used in the wet state for purposes not requiring the highest quality starch. By this process, all of the starch in the flour is recovered in a single fraction, the once-washed product usually having a protein content of between about 0.3 and 0.6%.

Protein obtained from wheat is of particular value at the present time for the production of monosodium glutamate which is much in demand as a condiment. Tests by a commercial producer of glutamic acid have shown that the by-product wheat protein from the alkali process is suitable for this purpose. Because of the chemical alteration which has occurred, the recovered protein cannot be used for the fortification of wheat flour in the manner that "native" or "gum" gluten is used.

The alkali process applied to flour provides a relatively simple method for producing starch by the use of conventional types of processing equipment. The recovery of precipitated protein and tailings starch appears to offer technical difficulties which would require study from a chemical engineering viewpoint for the large-scale application of the alkali process. The process, in certain cases, could be employed advantageously in conjunction with existing plant facilities. The production of sirups or sugars by the conversion of starch prepared from wheat or other cereal grain flour in beet-sugar factories or sugar refineries might merit consideration in regions where the raw material supply and cost are favorable. These establishments already have most of the equipment necessary for working up the starch conversion liquors. The utilization of beet-sugar factories in this way also would permit year-around operation of the plant by the production of starch conversion products during the idle period between sugar campaigns.

### Summary

As a basis for the production of starch from flour, conditions have been established for the essentially complete dispersion of the protein of wheat flour, without materially affecting the starch, by treatment with dilute aqueous sodium hydroxide. Precipitation of the protein on acidification of the alkaline solution has been studied.

Results of preparation of starch and by-product protein from wheat flour by alkali treatment have been presented. Two representative procedures, centrifuging and tabling, were used for starch separation. The alkali process, as used for wheat flour, has been applied also to barley, rye, oat, corn, sorghum, and rice flours. These raw materials, under the conditions used, were not as satisfactory as wheat flour.

The tabling process yields a prime-quality starch fraction, equivalent to 70 to 80% of the starch in the wheat flour. The remainder of the starch is recovered as a lower-quality fraction which is low in protein (0.4–0.7%) and suitable for certain conversion or fermentation uses.

The centrifuging process permits isolation of all the starch of the wheat flour in a single fraction having a protein content of 0.4–0.6%. Included in this product are the other alkali-insoluble solids which reduce the purity of the starch to between 94 and 98%.

The recovered by-product wheat protein has a purity of 70 to 90% and constitutes 50 to 80% of the flour protein.

The alkali process offers a means of utilizing any variety of wheat, as well as some other cereal grains, as alternatives to corn for maintaining or increasing the production of starch under special conditions of raw material supply and cost.

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## EFFECT OF THE SULFUROUS ACID STEEP IN CORN WET MILLING

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In the wet-milling process for the manufacture of corn starch it is customary to steep whole corn kernels, prior to milling, in dilute sulfurous acid solution for from 38 to 60 hr, depending upon the moisture, age, and type of corn. Too much emphasis cannot be placed upon the importance of steeping for ensuring smooth milling operation and maximum yield of prime-quality starch. According to Bartling (1940) the chief purposes of the steep are: (1) to soften the corn kernel, (2) to reduce or inhibit the activity of microorganisms, and (3) to whiten the starch. The softening action is apparently of the greatest importance. Regarding the mechanism of the action of aqueous sulfur dioxide little or nothing is known, although it has been widely assumed in the industry that dispersion of the protein is facilitated (*cf.* Brössler, 1895) with consequent loosening of the starch granules from the surrounding proteinaceous material. As far as is known, however, no studies have been reported on the function of sulfur dioxide in the steep.

Information on the specific action of the sulfurous acid in the steep would provide a logical basis in the search for superior steeping agents or for developing new processing methods. Experiments were therefore undertaken having three main objectives: (1) to determine the degree to which sulfurous acid in the steep aids in the softening of corn kernels, (2) to ascertain the starch-protein relations within the corn kernel, and the effect of the sulfurous acid steep upon them, and (3) to determine what, if any, effect the concentration of sulfurous acid in the steep has upon the starch subsequently extracted from the corn.

Since direct physical or chemical methods for following softening action are lacking, the simplest and most satisfactory approach ap-

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peared to be by microscopic examination of changes produced under various steeping conditions. This method was used and the results correlated with those of starch extraction studies.

### Experimental and Discussion

*Corn Endosperm.* Endosperm cells vary greatly in size. Just under the aleurone layer the storage cells in the dent corn (Iowa 939) studied were found to measure approximately  $50 \times 35 \mu$  (radial diameter  $\times$  tangential diameter, respectively). (Cf. Fig. 1.) Only about 2 to 8 rows of cells were this small; centripetal to these lay others, very irregular in size, ranging from approximately  $80 \times 50 \mu$  to  $240 \times 100 \mu$  (Fig. 2). In some kernels no further differentiation was observed, but in others there was a row of flattened cells at the center of the kernel, approximately  $55 \times 70 \mu$  to  $50 \times 150 \mu$ . In sectioning, some cells are cut through the center while others are cut more or less toward one end; hence cells having the same over-all dimensions may appear to differ considerably in size when the cross-sectional diameters are compared, as in Figures 4, 6, 7, and 8. Cell size also varies somewhat from kernel to kernel. Measurements of cell-wall thickness were made on cross sections of dent corn kernels mounted in distilled water. The average in the horny endosperm was 2 to  $5 \mu$ . Cells just under the aleurone had the thickest walls. In commercial starch processing, this cell-wall material contributes heavily to the "fiber" fraction, which is removed by the silk screens prior to the collection of prime-quality starch.

Endosperm cells showed 30 to 85 starch granules exposed by cross sectioning, the number of granules varying with cross-sectional diameters of cells. Small granules in a heavy protein matrix occurred just under the aleurone layer. Nearer the center of the kernel the granules were much larger in size and the protein network less massive. The difference in size of the starch granules is strikingly shown by comparison of Figures 1 and 2. The granules immediately under the aleurone layer are of the smallest size. Within a more or less narrow region, the granules are larger in diameter the greater their distance from the aleurone layer. Granules 3 to 9 cells distant from the aleurone layer are about equal in size to those in cells near the center of the kernel. Most of the starch granules lying close to the aleurone layer are contributed during processing to the so-called "tailing" or "bastard" starch, and relatively few to the prime-quality fraction.

Relationships between dent corn endosperm and embryo are shown in Figure 3. For details of kernel structure, see Hayward (1938).

*Penetration of the Kernel by the Steep.* Several steeps were made in water and in sulfurous acid from which samples of dent corn were with-

drawn at short intervals and examined macroscopically after sectioning to determine the site and degree of penetration of the steep liquor into the kernel. The liquid entered at the chalazal or basal end of the kernel

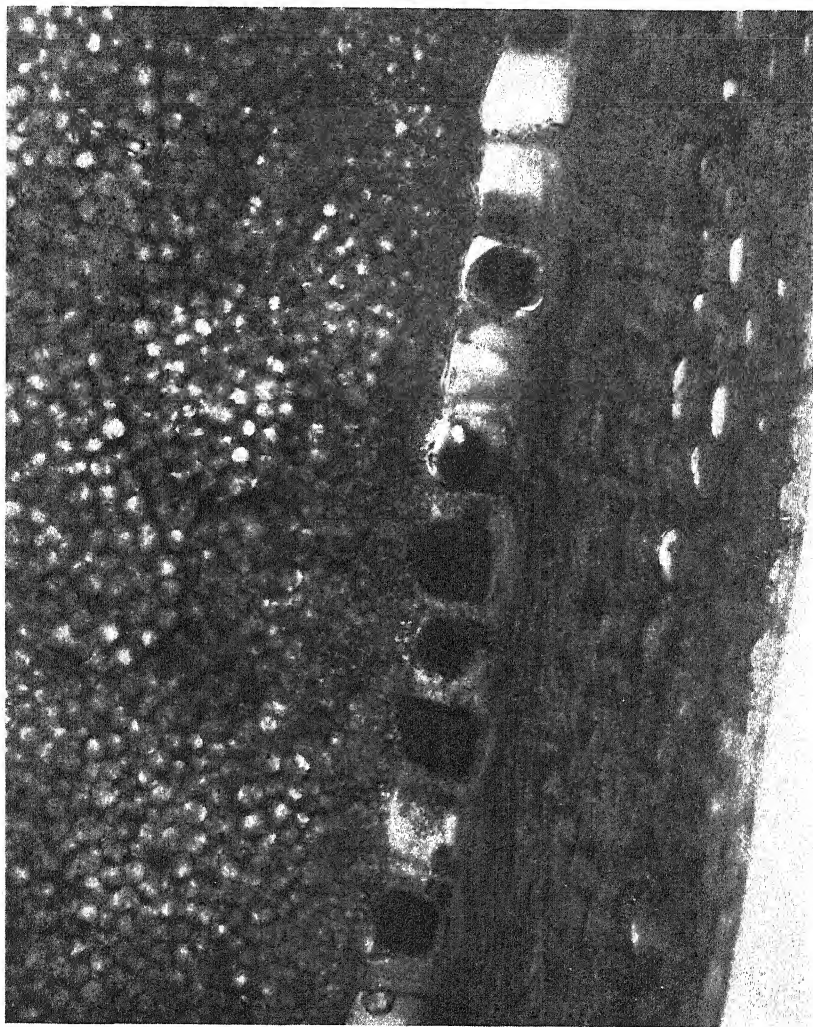


Fig. 1. Cross section approximately midway between base and crown of corn kernel, showing pericarp, aleurone cells (from some of which the contents have been lost in sectioning), and outer endosperm storage cells full of starch granules. 500 X.

and traveled rapidly upward just under the seed coat. It apparently thus reached the top of the kernel before starting to penetrate through the aleurone layer to the starch cells of the endosperm at the sides to



any appreciable extent. The embryo including the scutellum was penetrated before the endosperm. Somewhat less than 4 hr was required at 49°C for complete wetting of the germ and about 8 hr for complete penetration of the endosperm by the steep. At the end of

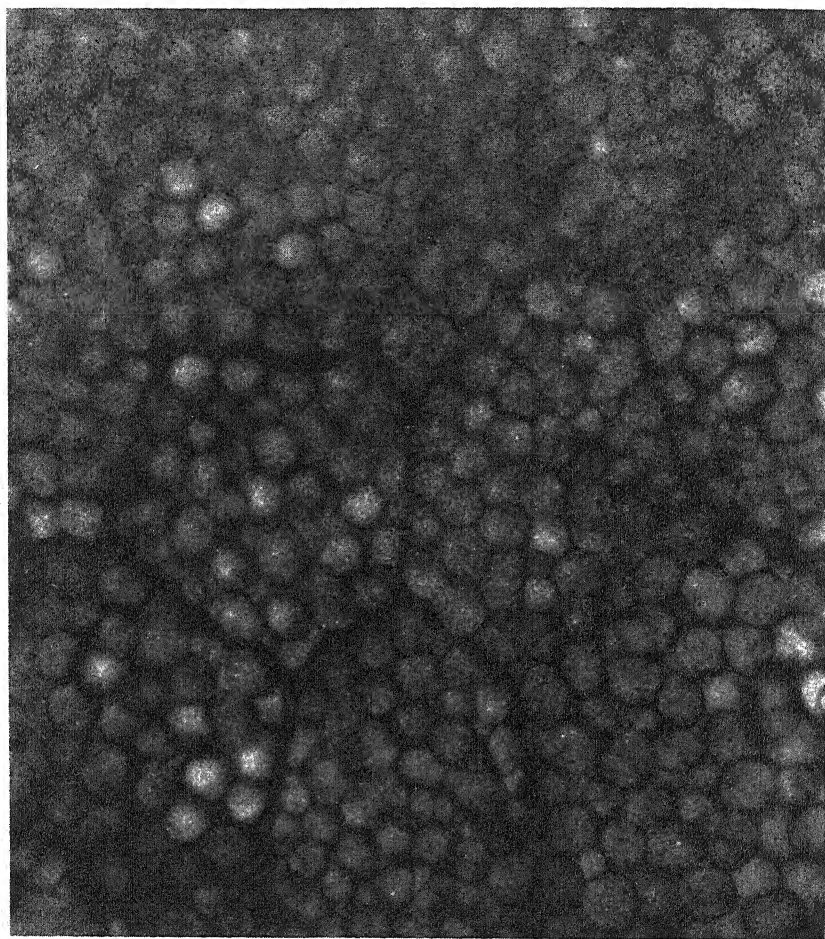


Fig. 2. Cross section near inner edge of horny endosperm, approximately midway between base and crown, showing endosperm storage cells full of starch. 500 X.

8 hr, however, the kernel was not greatly softened. Corn became progressively easier to grind as the duration of the sulfurous acid steep was increased from 4 to 24 hr.

*Starch-Protein Relations.* Sections of corn kernels were obtained for microscopic studies by use of a sliding microtome with freezing

attachment. When these sections were stained directly with iodine solution none of the details of the starch-protein relations could be observed owing to the opaqueness of the deeply stained starch granules. The following method, therefore, was devised for removing the starch granules from a section of corn kernel and at the same time staining the protein network and the cell walls: The section was placed in a drop of distilled water on a microscope slide, flooded with 0.2*N* iodine solution, and allowed to stand  $\frac{1}{2}$  min; the iodine was pipetted off;

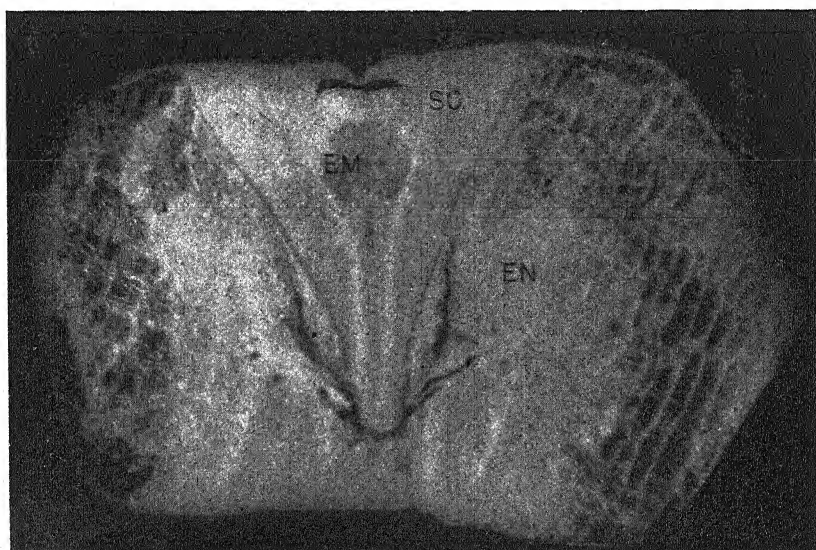


Fig. 3. Cross section through the middle of corn kernel from which the pericarp, commonly known as the seed coat or hull, has been removed. Positions of the embryo (em), including the scutellum (sc), and of the endosperm (en) are indicated. The floury endosperm near the scutellum has been torn slightly in sectioning. The cells show clearly in the horny endosperm, but are less distinct in the floury. Approximately 13 X.

and a drop or two of 70% sulfuric acid was added. After about 1 sec the sulfuric acid was rinsed off with distilled water and the section washed several times to remove the starch. The sulfuric acid gelatinized the starch granules and, in consequence, they were easily removed from the surrounding protein which remained very tough and hard to break. A drop or two of 0.2*N* iodine solution was then placed on the moist section and the cover slip added. The initial treatment with iodine and sulfuric acid left the cell walls unstained or stained blue depending upon the amount of cellulose present, while the final addition of iodine gave a bright yellow color, characteristic of protein, to the network. At the same time, any starch not removed by the previous washes was colored deep blue-black.

Comparisons were made of sections stained in this manner with others prepared: (1) by mounting directly in cold water, (2) by heating in water to gelatinize the starch granules which thereupon were forced out of the protein network, (3) by removing all loose starch granules by washing with water and subsequently staining the protein network

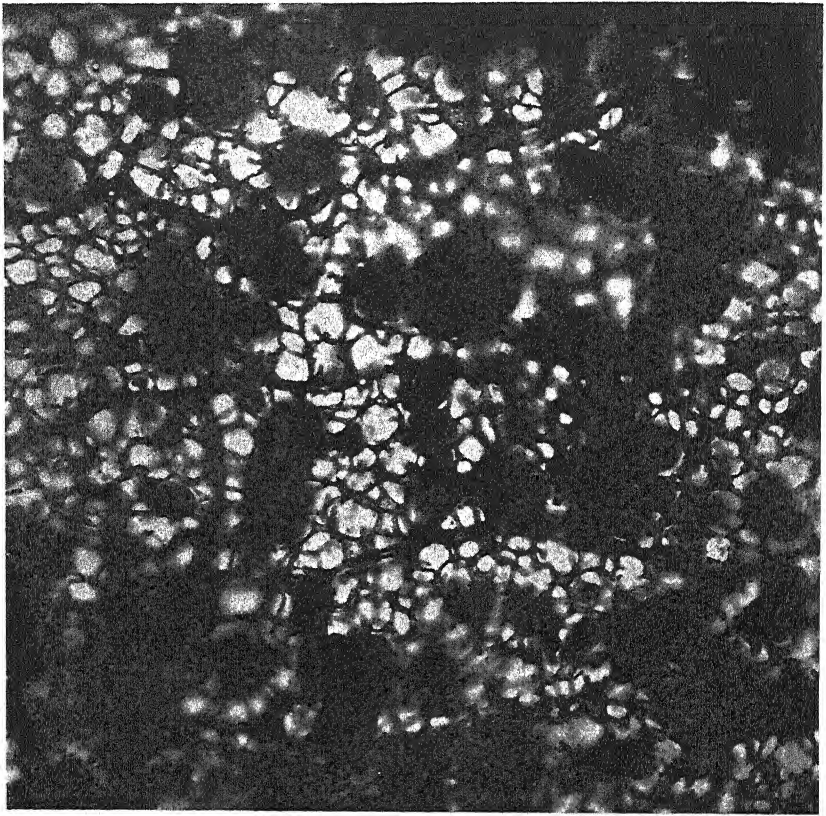


Fig. 4. Cross section of unsteeped dent corn, comparable to that shown in Figure 2 as to position, but with the starch granules gelatinized and largely removed. The proteinaceous network holds them tightly. Those remaining in the network appear as large black bodies having been stained with iodine. The thick dark lines are cell walls; the thinner lines in honeycomb arrangement are the protein network. 500 X.

with picric acid, and (4) by the use of several combinations of stains commonly employed on plant material. There was no evidence that the microscopic details and relationships of protein, starch, and cell walls were in any way changed by the treatment adopted as standard, other than that the starch was gelatinized and consequently often forced out of the protein network.

Careful microscopic study of cross sections of Iowa Hybrid 939 dent corn endosperm showed that the individual starch granules in both the horny and the floury endosperm lay completely embedded in a matrix composed largely, if not wholly, of protein. In the floury endosperm at the center of dent corn kernels the protein is less dense and each protein envelope fits about the enclosed starch granule much more loosely than in the horny endosperm. When the starch granules were

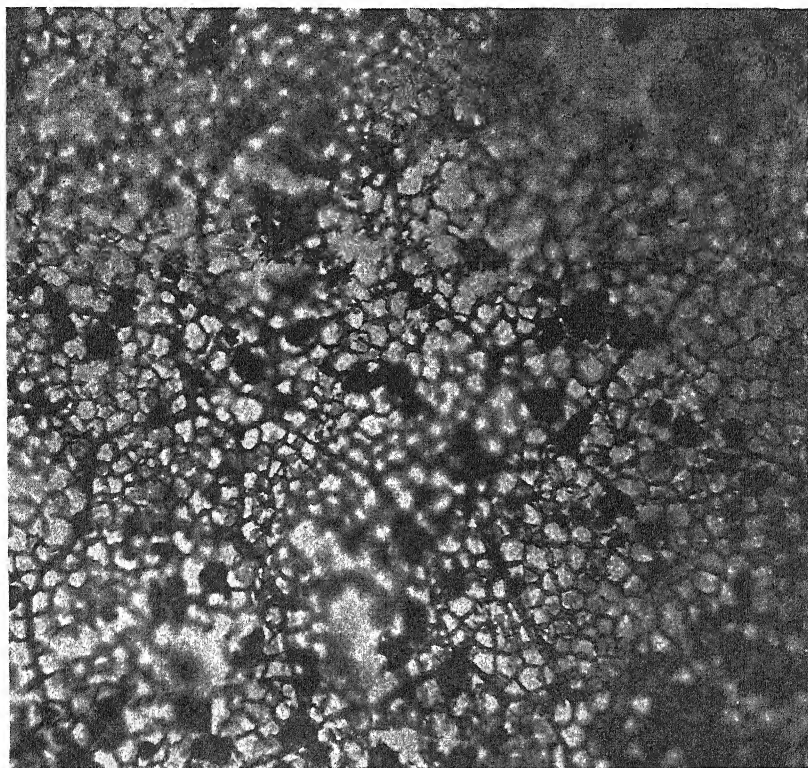


Fig. 5. Cross section of unsteeped popcorn, comparable to that of dent corn shown in Figure 4. The protein network is stronger than in dent corn and has been more completely freed of starch granules without causing damage to the network. Some starch granules, however, still remain. 200  $\times$ .

removed from thin sections (as described above), the empty protein network was clearly visible within the individual cells (Figs. 4 and 5).

In Cuzco, the flour corn studied, there is a heavy protein network just inside the aleurone. This is, however, less dense than in the corresponding area of dent corn. In Cuzco corn the protein matrix becomes progressively weaker from the aleurone to the center where it is found largely in discrete flat particles instead of as a network.

The strands of protein composing the network were found to be birefringent between crossed nicol prisms, indicating that the majority of the molecules in the network, that is, in the protein film, are oriented. In crystalline or oriented high polymeric polar substances it is generally recognized that powerful intermolecular binding forces are operative. As a result, such high polymers are usually strong and difficult to disperse in solvents. The oriented and therefore possibly crystalline nature of the protein network probably accounts for its toughness and for the difficulty encountered in dispersing it. At a magnification of  $1200\times$  the protein appeared homogeneous, without structural detail.

From the microscopic studies it appeared that the protein content was considerably greater in horny than in floury endosperm. This was indirectly confirmed by analytical data obtained previously, and for another purpose, on hand-separated horny and floury endosperm fractions of another typical dent corn, Reid Yellow Dent. These data are summarized in Table I. The 4 to 5% unaccounted for by analysis

TABLE I

COMPOSITION OF HORNY AND FLOURY ENDOSPERM OF REID YELLOW DENT CORN

Constituent	Horny endosperm	Floury endosperm
	%	%
Ash	0.27	0.35
Protein ( $N \times 6.25$ )	13.25	7.69
Alcohol soluble N	1.49	0.64
Sugar	0.47	0.52
Starch (by polarimetric method)	80.4	85.6
Oil (petroleum ether extract)	0.73	0.33
Undetermined	3.4	4.9

is undoubtedly attributable to cell-wall material. From these data it is apparent that there is almost twice as much protein in horny as in floury endosperm. The ratio of starch to protein is approximately 11 : 1 in floury and 6 : 1 in horny endosperm. Although the microscopic and analytical studies were made upon different varieties of corn, dent corns, in general, appear to be sufficiently similar to warrant correlation of these data.

*Correlation of Microscopic and Extraction Studies.* Three corns were chosen for the microscopic investigations on steeping. *Iowa Hybrid 939*, a dent variety very popular in the corn belt, grown in Iowa, was used as typical of the type of corn reaching wet millers; it contains both horny and floury endosperm. *White Cuzco* flour corn, grown in Peru, and *South American* variety popcorn, grown in Iowa, were used in a study of possible differences in the effects of steeping on floury and horny endosperm, respectively.

For more detailed studies of the effect of steep upon starch characteristics, *Iowa Hybrid 939* and *Iowax 1*, comparable hybrid corn into which the waxy or glutinous starch character has been bred, were used. The latter was chosen because of wide current interest in glutinous starch for tapioca replacement.

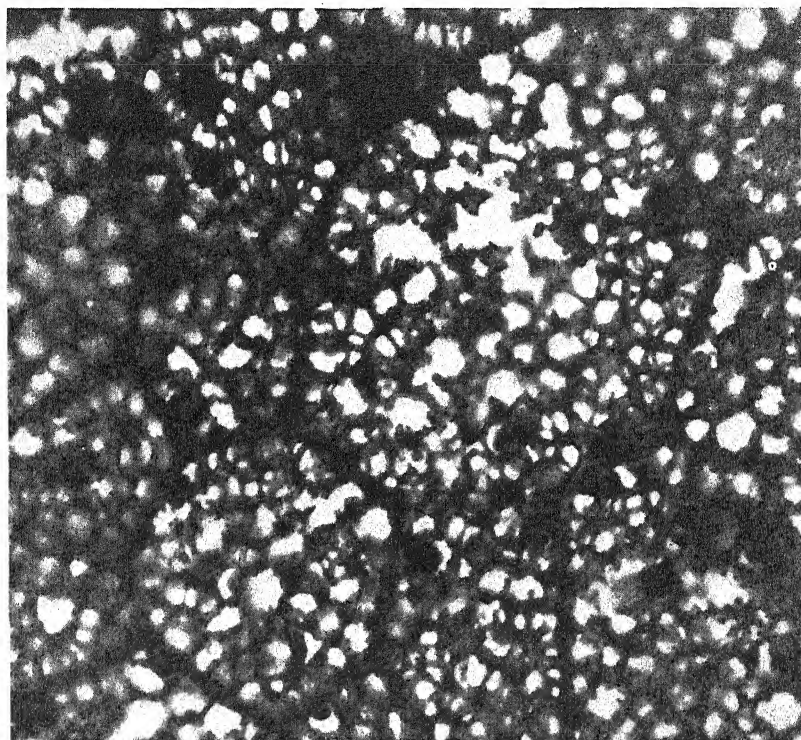


Fig. 6. Cross section of corn kernel comparable to those shown in Figures 4 and 5. Dent corn after 24-hr steep in distilled water. The protein network is somewhat swollen; in parts of the network incipient globule formation is apparent. 500 X.

Starch was extracted on a laboratory scale by a method analogous to that used commercially for the production of corn starch. Although practice varies considerably from plant to plant, most, if not all, of the wet millers in this country use steeps having sulfur dioxide concentrations of between 0.10 and 0.30%. In order to cover this range and to study more extreme conditions as well, the present experiments were carried out with distilled water and 0.10, 0.20, 0.30, and 0.40% sulfur dioxide steeps. A 24-hr steeping period at 49°C was adopted as standard. In certain instances, as noted below, other steeps were used for special studies. Steeping was carried out by placing 400 g of corn and



1700 ml of steep liquid in a 2-l Erlenmeyer flask in a thermostatically controlled bath. The bottom of the flask had been drawn downward into a shallow inverted cone. Steep liquid was withdrawn from the bottom at the center of the flask (*i.e.*, the lowest point) and returned through a circulating pump to the top of the flask. The steeping system was entirely closed, except for a small vent through the flask stopper. The bath temperature was recorded automatically throughout the steeping period. In sulfurous acid steeps, the sulfur dioxide

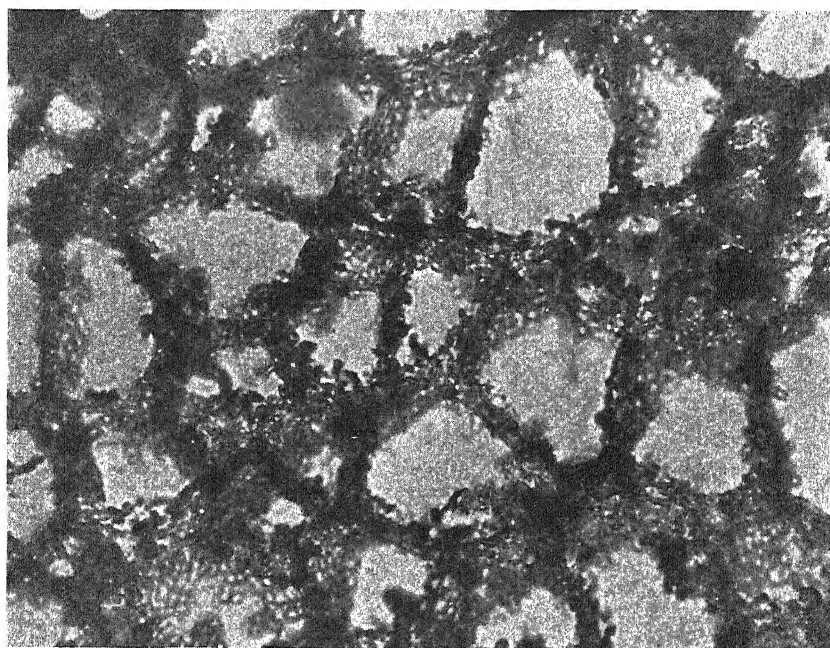


Fig. 7. Cross section of dent corn kernel, comparable to those shown in Figures 4 and 6, after a 24-hr 0.2%  $\text{SO}_2$  steep. The cells appear smaller than those in the other figures only because of variations between individual corn kernels. Disintegration of the protein matrix has proceeded so far that the network breaks considerably when the starch granules are removed; the weakened network has collapsed onto the cell walls. 500  $\times$ .

content decreased to about one half its original value during a 24-hr period. Since the effect of only one steep constituent was under investigation, no attempt was made to duplicate a commercial steep, which employs gluten overflow water containing appreciable quantities of substances ("solubles") dissolved from the corn.

After decantation of the steep liquor, the corn was ground through a Hobart sausage mill, screened through No. 17 silk bolting cloth, and the starch collected by centrifuging. This crude starch was separated into prime-quality and "tailings" fractions by resuspending in distilled

water, centrifuging, and scraping the "tailings" fraction layer from the surface of the starch. Washing and separation were repeated 4 to 5 times, or until no layering was obtained on centrifuging. The prime-quality starch was dried in a forced draft oven at 40°C.

Analytical methods and details of the procedure for viscosity determinations have been described elsewhere (MacMasters and Hilbert,

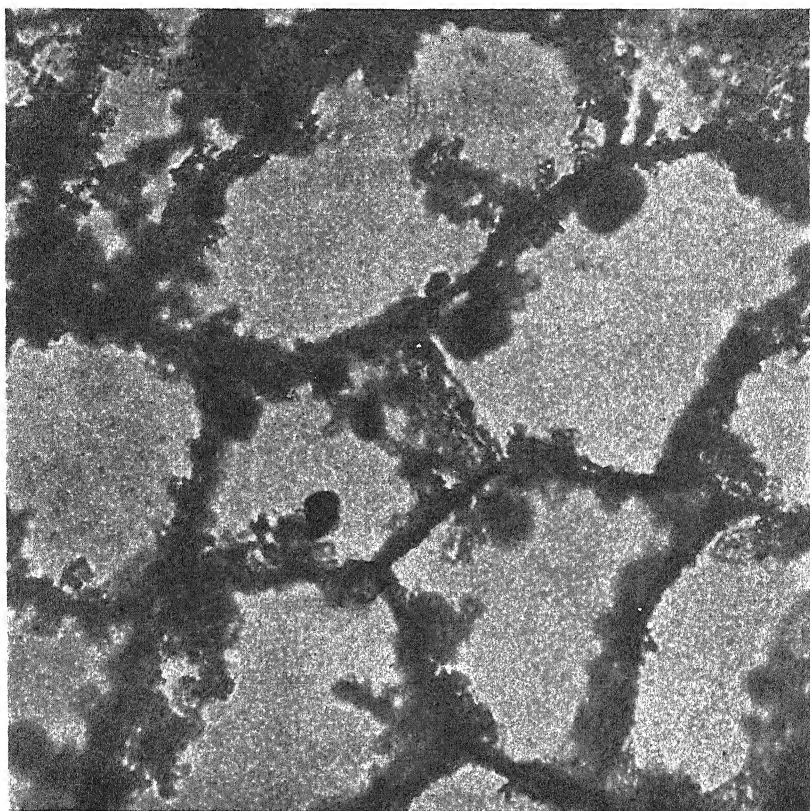


Fig. 8. Portion of cross section of dent corn kernel after a 24-hr 0.4%  $\text{SO}_2$  steep. The protein matrix is almost completely disintegrated; the remaining pieces have dropped out during preparation of the section and only those supported by the cell walls are evident. 500  $\times$ .

1944). Viscosities were taken with a Stormer viscometer, and hence are only qualitatively comparable with commercial viscosity data.

After dent corn had been steeped in sulfur dioxide solution, the protein matrix was found to be more or less altered, the degree of change depending upon the concentration of sulfur dioxide in the steep. When distilled water or 0.10% sulfur dioxide steep was used, the protein network was still almost as distinct as originally and apparently almost as



strong. (Cf. Figs. 4 and 6.) The network was more swollen, however, and its individual portions often appeared to be made up of tiny globules of hydrated protein. After a 0.20% sulfur dioxide steep, the network was appreciably weakened and partially dispersed. Upon removal of the starch granules, the undispersed protein usually collapsed against the cell walls and appeared as a mass of material with little evidence of the original network films. This is shown in Figure 7. After a 0.4% sulfur dioxide steep, much of the protein had been dispersed, and only a relatively small amount lay along the cell walls after the starch had been removed (Fig. 8). These data are summarized in Table II. They refer to the greater part of the endosperm;

TABLE II  
EFFECT OF NATURE OF STEEP ON STARCH-PROTEIN MATRIX RELATIONS  
AND ON RECOVERY OF STARCH

Steep		Condition of protein in matrix	Starch recovery
Type	Duration		
	hr		%
None	—	Coarse network, holding starch tightly	—
Distilled water	4	Slightly less coarse, but holding starch tightly	—
Distilled water	24	Slightly less coarse than at 4 hr, but holding starch tightly	64
0.1% SO <sub>2</sub>	4	Slightly less coarse than unsteeped, holding starch tightly	—
0.1% SO <sub>2</sub>	24	Somewhat less coarse than at 4 hr, holding starch tightly	82
0.2% SO <sub>2</sub>	8	More dispersed than after 24-hr steep in 0.1% SO <sub>2</sub>	—
0.2% SO <sub>2</sub>	24	Not appreciably different from 8-hr sample	83
0.3% SO <sub>2</sub>	4	Much dispersed	—
0.3% SO <sub>2</sub>	24	In separate particles; no network remaining	88
0.4% SO <sub>2</sub>	4	Indistinguishable from 4-hr steep in 0.3% SO <sub>2</sub>	—
0.4% SO <sub>2</sub>	24	Indistinguishable from 4-hr sample	89

just under the aleurone layer, however, the protein matrix appeared more dense and did not disperse so readily. In this region, only hydration, comparable to that shown in Figure 6, was observed after all steeps except the 0.4% sulfur dioxide. After this steep many globules could be seen in the network.

Disintegration of the protein matrix was more rapid in Cuzco corn than in dent (cf. Figs. 8, 9, and 10), and slower in popcorn. This would be expected from the comparative amounts of protein present.

Several 0.2% sulfur dioxide steeps were run at temperatures below (38°, 43°) and above (52°, 55°) 49°C to determine what effects temperature of the steep might have. Variations in starch recovery were not

significant under laboratory processing conditions although processing was easier the higher the temperature used. It is well known by the industry, however, that the temperature of the steep employed at the factory has a profound effect upon both the ease of processing and the amount of starch recovered.



Fig. 9. Portion of cross section of Cuzco corn kernel after a 6½-hr 0.4% SO<sub>2</sub> steep. The protein network is still strong; globule formation is evident. 200 X.

Microscopic examination showed the protein to be largely in globules after the corn had been steeped at 38° or 43°C. Just under the aleurone layer, only hydration of the protein, with consequent swelling, was evident. After steeping at 52° or 55°C, the protein was largely dispersed; in the cells just under the aleurone layer many protein globules were observed.

When the sulfur dioxide concentration was increased to 0.4%, steeping at 52°C dispersed the protein in most of the endosperm almost completely; in the cells just under the aleurone layer considerable dispersion had occurred, but many globules were still present.



Fig. 10. Portion of cross section of Cuzco corn kernel after a 24-hr 0.4%  $\text{SO}_2$  steep. The protein network is largely disintegrated and dispersed; remaining portions have fallen back against the cell walls. Note that the three-dimensional character of the network is clearly evident in this picture. 200  $\times$ .

The apparent sequence of protein hydration, formation of globules, and subsequent progressive disintegration suggests that the formation of the globular bodies represents an early step in the disintegration of

the protein network, followed by breakage into small fragments, with some of the fractions going into ultimate colloidal dispersion.

From the data, it is evident that both high sulfur dioxide concentration and high steeping temperature lead to increased protein disintegration and dispersion. The relationship between protein dispersion and starch recovery is probably due to the fact that the more disperse the protein the less starch it can carry into the tailings or gluten fraction, and, hence, the greater the amount of prime-quality starch left on the table or in the centrifuge. These results on the disintegration of protein by sulfur dioxide are corroborated by the behavior of the gluten fraction from water-steeped corn kept overnight at 49°C in 0.2% sulfur dioxide and in distilled water. In both instances the protein was still in microscopic aggregates, but after the sulfur dioxide steep these were of only about one fourth the diameter of those remaining after the water steep. These partially disintegrated aggregates were found to be birefringent.

In order to determine whether the softening action of sulfurous acid upon the corn kernel might be due to acidity, steeps were run using acetic and hydrochloric acids, respectively, in concentrations equivalent to a 0.2% sulfur dioxide solution. Corn steeped in acetic acid solution was somewhat hard to grind, and separation of starch from gluten was difficult. A 70% recovery of starch, containing 0.10% nitrogen (dry basis), was obtained. After the hydrochloric acid steep, the corn was ground with difficulty. Separation of starch from gluten was fairly easy, but only a 56% recovery of starch, containing 0.07% nitrogen (dry basis), was obtained. The relatively low yield of starch was in part due to the difficulty in grinding the horny endosperm to a finely divided condition. As a result, there were many large particles of horny endosperm screened away from the crude starch.

Similar results were obtained when acetic and hydrochloric acid steeps at pH 1.68 to 1.70, equivalent to that of 0.2% sulfur dioxide, were used. A corn sample steeped only 4 hr in sulfurous acid solution was as easy to process as that steeped for 24 hr in either acetic or hydrochloric acid solution. It was concluded that acidity can play no more than a minor role in softening the corn kernel and loosening the gluten from around the starch granules.

The action of lactic acid as a steeping agent was also studied, since during industrial steeping a considerable amount of this acid is formed by microbial action and is believed by some industrialists to exert a favorable action in the steeping process. Lactic acid in 0.1% concentration effected protein disintegration approximating that brought about by a water steep, and softening roughly equal to that found after a 0.1% sulfur dioxide steep. Both 0.2% and 1% lactic acid steeps

produced protein disintegration about equal to that given by a 0.1% sulfur dioxide steep, and softening approximating that produced by a 0.2% sulfur dioxide steep. A steep containing 0.1% sulfur dioxide and 0.1% lactic acid effected protein disintegration about equal to that resulting from a 0.2% sulfur dioxide steep, but produced slightly more softening than the latter. After the steep containing 0.1% sulfur dioxide and 0.1% lactic acid, the degree of protein disintegration was equal to that produced by a 0.1% sulfur dioxide and 0.2% lactic acid steep, but softening was considerably greater in the latter. Lactic acid has relatively little ability to disintegrate the protein matrix but does exert an appreciable influence in softening the corn kernel. The mechanism of its action is at present inexplicable.

Since the action of sulfur dioxide is not due to its acidity, it might be assumed to be due to its reducing action, especially since sulfites and mercaptans are known to have considerable dispersing action on some proteins. Olcott, Sapirstein, and Blish (1943) have shown that reducing agents have a double action in wheat glens and flours, first a chemical action on the protein molecule, and second, an activation of the native proteinases present. It is reasonable to expect a similar double effect in corn. The action of reducing agents other than sulfurous acid on the corn protein matrix is under investigation.

*Steeping Studies on Artificially Dried Corn.* It is common knowledge among corn wet millers that great difficulty is experienced in processing corn artificially dried at 180° to 200°F, apparently because of changes brought about in the endosperm. A sample of corn dried under these conditions was steeped in a 0.2% sulfur dioxide solution for 24 hr at 49°C. The strands in the protein network appeared slightly narrower in the dried corn and upon steeping did not swell to the same extent as those in corn not artificially dried. The starch was, moreover, held more tenaciously by the protein matrix in the artificially dried sample. Protein dispersal during steeping appeared to be nearly normal, except that the disintegrating matrix left pieces which adhered closely to the starch granules. Upon extraction it was impossible to separate all of the gluten from the starch and a prime-quality starch was not obtained.

A second sample of corn, artificially dried at 120°F, appeared intermediate in behavior between corn dried in the field and that dried at 180° to 200°F.

*Effect of Steep on Starch.* Data showing the effect of the sulfur dioxide concentration of the steep upon noncarbohydrate constituents and viscosity of glutinous and nonglutinous corn starches are summarized in Table III. Nitrogen, or protein, and phosphorus content increased as sulfur dioxide concentration of the steep increased; the

TABLE III  
EFFECT OF SULFUR DIOXIDE CONCENTRATION OF STEEP UPON  
COMPOSITION AND VISCOSITY OF STARCHES  
(All steeps were run at 49°C for 24 hr)

Type of corn starch	SO <sub>2</sub> concentration of steep		On vacuum-dried basis				Relative viscosity <sup>1</sup>	
	Initial	Final	N	P	Ash	MeOH extd. fat	Initial	10 min
Nonglutinous (Iowa hybrid 939)	%	%	%	%	%	%	3.5% paste	
	0.00	0.00	0.03	0.013	0.06	0.64	7.1	6.1
	0.1	0.05	0.04	0.016	0.06	0.53	5.1	4.0
	0.2	0.1	0.05	0.020	0.05	0.59	4.6	4.5
	0.4	0.2	0.06	0.021	0.06	0.70	3.6	3.2
Glutinous (Iowax 1)							2% paste	
	0.00	0.00	0.03	0.006	0.03	0.20	4.2	2.5
	0.1	0.06	0.03	0.009	0.04	0.16	2.4	2.1
	0.2	0.1	0.05	0.013	0.05	0.20	2.5	2.0
	0.4	0.2	0.08	0.014	0.05	0.24	1.7	1.6

<sup>1</sup> Glutinous starch has a much higher viscosity than nonglutinous; hence, viscosity determinations were made on 2% pastes of the former and 3.5% pastes of the latter. The initial and 10-min values are those obtained immediately after placing the paste in the Stormer viscometer and after the eleventh reading taken at the end of 10 min, respectively.

amount of methanol-extractable fat, however, remained relatively constant. In the nonglutinous corn starch the ash also remained constant, but in the glutinous it increased with increasing sulfur dioxide concentration of the steep.

Starch extracted from either glutinous or nonglutinous corn after a 0.1% sulfur dioxide steep was appreciably lower in viscosity than that extracted after a water steep. The higher the sulfur dioxide concentration of the steep, from 0.1 to 0.4%, the lower was the viscosity of the starch extracted. Viscosity tended to vary inversely with the nitrogen and phosphorus contents of the starches. Sulfur dioxide concentration of the steep was not found to have any appreciable bleaching effect upon the starch.

### Summary and Conclusions

A method has been developed for following *in situ* changes occurring in structural protein when corn kernels and, presumably, other cereals are subjected to various conditions. By utilization of this method, it has been found that sulfurous acid in the steep used in the wet-milling process for corn starch extraction has a specific effect in facilitating the separation of starch from protein.

A technique has been developed whereby the starch granules can be

removed from a section of the kernel, leaving the protein network clearly visible. Birefringence of this network indicates that the molecules in it are largely oriented.

In corn kernels the starch in the endosperm cells lies embedded in a proteinaceous matrix. The smaller starch granules and protein predominate in the cells just under the aleurone layer, while larger granules and less protein lie nearer the center of the kernel. In horny endosperm, in general, the protein content is about twice that of the floury endosperm.

Considerable peptization of the protein is effected when corn kernels are subjected to a sulfurous acid steep. At 38° to 43°C, a 0.2% sulfur dioxide steep leaves much of the protein in globules or in strands ending in globules. At 49° to 55°C greater dispersion of the protein results from steeping, and globules are found only in the cells just under the aleurone, where protein packing is most dense. The steep causes more complete protein dispersion, the higher the temperature (38° to 55°C), and the greater the sulfur dioxide content (0 to 0.4%).

Sulfurous acid apparently effects disintegration and dispersal of the protein matrix more by its reducing action than by its acidity. It cannot be successfully substituted by lactic, acetic, or hydrochloric acid in equivalent concentration, or by acetic or hydrochloric acid at the same pH. Addition of lactic acid to a sulfurous acid steep increases softening of the kernel.

Both glutinous and nonglutinous corn starch contain more nitrogen and phosphorus and have lower paste viscosity the higher the sulfur dioxide content of the steep used prior to extraction (from 0 to 0.4%). In glutinous corn starch, the ash content also is greater. The fat content of the starch is not affected by the steep.

It is difficult or impossible to separate prime-quality starch from corn dried artificially at high temperatures, since the protein adheres to the starch granules with unusual tenacity. The difficulty is greater the higher the temperature at which the corn has been dried.

The specific action of sulfurous acid in the steep is to disintegrate the protein matrix throughout the kernel, and, in consequence, to facilitate complete hydration with consequent softening of the kernel. This action is suggested to be due mainly to the reducing action of the sulfurous acid. Bleaching of the starch by the sulfurous acid during steeping is apparently negligible.

#### Acknowledgments

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## EFFECT OF PROTEIN CONTENT AND GRADE ON FARINOGRAMS, EXTENSOGAMS, AND ALVEOGRAMS <sup>1</sup>

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Machines designed to measure the physical properties of doughs are used in many cereal laboratories. In Great Britain and in continental Europe, the Farinograph and Extensimeter are used extensively for mill control on wheats imported for blending, and it is claimed that the machines provide useful information unobtainable by other means. In Argentina, the government authorities place considerable importance on the physical characteristics of the dough in establishing their standard wheat samples, and reproductions of Farinograms and of Alveograms for different grades are prominently displayed in their crop bulletins. In North America, cereal chemists rely largely on baking tests and subsidiary analyses for evaluating wheat and flour quality, although an increasing interest in dough-testing machines is apparent.

It would perhaps be generally agreed that dough-testing machines, apart from the recording type of dough mixer (Farinograph and Mixograph), have not been investigated widely enough to demonstrate their utility for assessing the quality of North American wheats. In Canada this lack of interest is not surprising, for Canadian wheat owes its position in world markets to its strength, a property that can be evaluated adequately by baking tests. Nevertheless, the widespread use

<sup>1</sup> Paper No. 68 of the Grain Research Laboratory and No. 229 of the Associate Committee on Grain Research (Canada).



of dough-testing machines by importers of Canadian wheat made it advisable for the Board of Grain Commissioners' laboratory to acquire, in addition to the Brabender Farinograph, a Brabender Extensograph and a Chopin Alveograph, and studies of these machines were started some years ago. This paper deals primarily with the effect of protein content and grade on the types of curves produced. The work was done in 1940 with a comparatively small number of samples, and it was the intention to repeat it with a larger number of samples in order that more adequate correlation studies might be made. However, as opportunity for repeating the studies has not presented itself, it now seems advisable to publish the original results.

The influence of protein content on Farinograph curves has been investigated in this and other laboratories. Aitken and Geddes (1938) reported that the dough characteristics measured by the Farinograph are not merely a reflection of protein content; for certain flours of the same protein content and loaf volume gave quite different types of curves. In a later study on gluten-enriched Canadian flours, the same authors (1939) modified this statement when they found that dough-development time and absorption increased with each increment in protein content. Markley (1938), in a study of the dough properties of flour-starch-water systems, also reported that dough-development time is a function of the amount of protein when this is over 7%, but that at lower levels there is no differentiation. Markley and Bailey (1939), in their statistical study of dough formation in an extensive and diverse series of flours, concluded that protein content is highly correlated with dough-development time and with absorption. Geddes, Aitken, and Fisher (1940) showed that dough-development time and curve consistency decreases were, respectively, positively and negatively correlated with protein content; and they also presented considerable data on Farinograms for different grades of Western Canadian wheat.

In an investigation of the Extensograph for predicting baking quality, Munz and Brabender (1940a) mention that there is some relation between protein content and area under the Extensogram. This statement was based on results for "artificial" flours, whose protein contents were either lowered by the addition of starch or raised by enrichment with wet gluten, and doubt was expressed as to whether such flours possessed properties that were identical with those of the original material. The scientific literature does not appear to contain any reference to the effect of protein content on Alveograms.

Investigation of the utility of the Farinograph, the Extensograph, and the Alveograph for evaluating the quality of Western Canadian wheat was begun several years ago, and some of the results obtained

have been referred to briefly in crop bulletins and in the annual reports of the laboratory. As a first step, the machines were used in routine studies of the comparative dough properties of average samples of different grade. Such samples provide a useful test of the machines, for they include wheats that are similar and others that vary widely in both protein content and baking quality. Moreover, their real values are well established and known to both domestic millers and millers in other countries. In general, the results showed that wide differences between certain grades were indicated by all the machines, but it appeared that these differences were closely related to protein content.

To obtain further information on the effect of protein content on Farinograms, Extensograms, and Alveograms, two special sets of samples were collected. These consisted of a series of wheats that varied widely in both protein content and baking strength, and a series of wheats that was essentially the same in these respects. These series, though small, were collected with considerable difficulty and are not easily duplicated. The present paper deals with the results obtained with these two sets of samples and with typical samples representing averages for different grades. The results for each series are discussed in separate sections.

### Methods

The Farinograph and Extensograph used in these studies are essentially the same as those described by Bailey (1940) in his comprehensive review of "Physical Tests of Flour Quality." The Alveograph is the redesigned Chopin Extensimeter which he also describes.

*Brabender Farinograph.* Two types of Farinograms were made, the normal curve and a high-speed curve. The procedure for the normal curve involved the use of a 50-g mixer and was the same as that outlined by Geddes *et al* (1940). Single Farinograms were made for each sample and dough-development time was the only measurement recorded. In this laboratory the standard error of the mean of duplicate curves is of the order of 0.3 min for this measurement.

The high-speed curve was developed by this laboratory in an effort to obtain greater differentiation among samples which usually show little or no differentiation by the normal curve. A 50-g mixer was modified by introducing additional gears so as to increase the speed of the mixing blades 2.3 times. The high-speed curve is made in the same manner as the normal curve, but the weight on the lever system is set in the position recommended for the 300-g mixer. This change is necessary to obtain a curve that falls within the range of the Farinograph scale. The dough consistency level corresponds to 400 Farinograph units. In evaluating high-speed curves, small deviations above

or below the 400 unit line are disregarded. On account of the rapid breakdown of the dough, 10 min mixing time is ample. The curve is interpreted in the same way as the normal curve, but there is a distinct break, and this affords an extra measurement.

The latest model of the Farinograph is equipped with a double-speed mixer; a normal speed of 60 rpm is provided for bread flours, and a slow speed of 30 rpm is provided for soft flours. According to the Brabender Corporation, slow-speed mixing was introduced to bring about gentle development of soft-flour gluten with minimum abuse of the dough. The principle is the same as that employed in this laboratory, but whereas we experimented with a higher speed to obtain faster development of strong flours, they use the reverse to obtain slower development of weak flours.

*Brabender Extensograph.* Prior to making a test, certain precautions must be taken with the dough mixer, and adjustment of the stretching and recording mechanism may also be necessary; otherwise faulty and misleading curves will be obtained.

The operating instructions supplied with the Extensograph state that all doughs must be mixed in a clean Farinograph mixer after one, and possibly two, cleaning doughs have been mixed and discarded. The explanation given for this is that a very fine layer of copper oxide forms on the surfaces of the mixer overnight, or on long standing, which greatly influences the curves. To determine the minimum number of cleaning doughs that are necessary, and also the time interval between tests when additional cleaning doughs are required, a preliminary study was made using various numbers of cleaning doughs, and with the mixer standing empty for different lengths of time. The results showed that after the mixer stood empty overnight and over a week-end, at least two cleaning doughs are necessary. After a 2-hr interval between mixings, one cleaning dough is required, but after a 4-hr interval two cleaning doughs again seem necessary. For safety, it is the standard practice in this laboratory to employ three cleaning doughs, each mixed for 5 min, before mixing a set of samples for Extensograph tests.

Proper centering of the dough hook is essential, and this can be done by means of the screw situated above it. The dough hook must be positioned in the center of the exposed portion of the dough when it first makes contact with it. If it is slightly to the left, or to the right, the dough may tear on one side when breaking and the length of the curve may be longer than when the dough breaks evenly on both sides.

The necessity for these precautions has been confirmed by Snodgrass<sup>2</sup> in his investigation of the reproducibility of Extensograms. In-

<sup>2</sup> Private communications from E. I. Snodgrass, Washington Mills, Glasgow, Scotland.

addition to finding that centering of the dough hook overcame the uneven tearing of the dough when it broke, he observed that several cleaning doughs were required to minimize the effect of the copper poisoning of the mixer. He considers that two cleaning doughs are adequate but additional cleaning doughs are necessary if the time between tests exceeds 5 min.

To conserve paper, and to facilitate comparison of curves, it is advantageous to turn back the kymograph paper on completion of each curve so that replicate curves, and those for each rest period, are superimposed. It is advantageous to start all curves from the same zero mark, and this can be facilitated by installing the mechanism shown in Figure 1 just above the contact that controls the kymograph. A trip

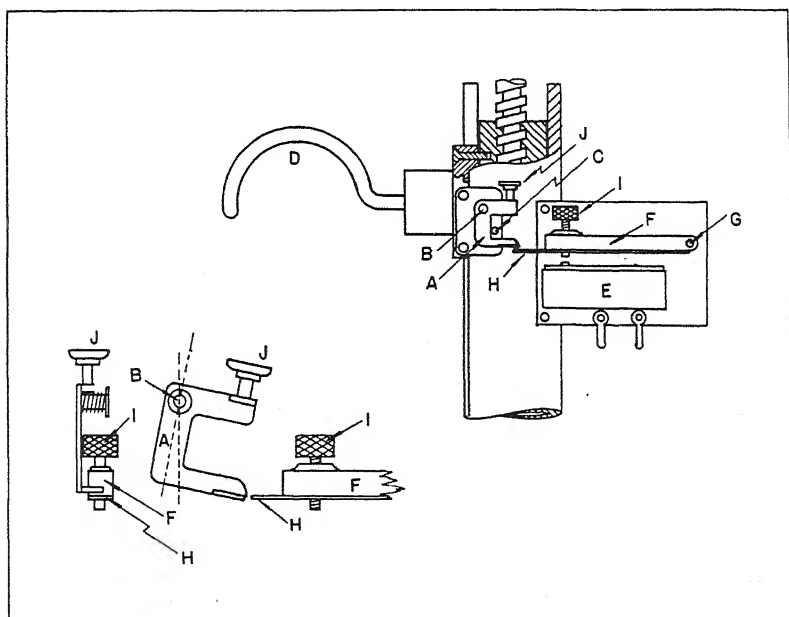


Fig. 1. Drawing of trip switch for Extensograph.

lever *A*, pivoted at *B* and with stop pin *C*, is attached to the dough hook *D*; and a normally closed, snap action, "micro-switch" *E* is attached to the column. The switch is actuated by an arm *F*, pivoted at *G*, and provided with a flexible extension *H* and a screw *I* for making fine adjustments in height. The descending hook is stopped just above the dough when the lever *A* engages the flexible extension *H* on the switch, provided to take care of the dying momentum of the hook after the motor is switched off. By pressing the button *J*, the lever and switch are disengaged (see detail) and the hook starts descending again.

This mechanism automatically stops the hook in the same position each time, and the kymograph paper can be adjusted so that the pen draws duplicate curves from the same starting point.

The Extensograph used in this laboratory has a paper velocity of 6.8 mm per sec and a hook velocity of 14.6 mm per sec. The tension on the balance corresponded to 200 units on the Extensograph paper with a balancing weight of 550 g.<sup>3</sup>

The routine testing procedure is the same as that outlined by Munz and Brabender (1940), with the exception that the absorption of the 300 g of flour is adjusted to 600 instead of 500 Farinograph units, and duplicate curves are made after a rest period of 135 min instead of after 60 min. (In some studies made in this laboratory, curves are also made after a rest period of 45 min.) Two curve measurements are made: maximum height (cm), which measures the resistance of the dough to extension; and maximum length (cm), which measures the extensibility of the dough from moment of first stretching to the final break. The standard error of the mean of duplicate curves is of the order of  $\pm 0.7$  cm for an average length of 20 cm, and  $\pm 0.2$  cm for an average height of 6.5 cm.

*Chopin Alveograph.* In this test a fixed absorption is recommended because adjusting the absorption to suit a particular flour frequently causes the curve to run off the kymograph paper. Kent-Jones (1939) states that testing doughs at similar consistencies (variable absorption) does not alter the order of evaluation of flours, though differences between strong and weak flours may be reduced. Although the absorption level varies with different classes of flour, 48% has been found satisfactory for flours milled experimentally from Western Canadian wheat. Dough consisting of 250 g flour (13.5% moisture basis) and sufficient 2.5% sodium chloride solution to bring the absorption to 48% is mixed for 6 min in the "Petrin extracteur." The dough is then extruded and cut into five discs of uniform size. The discs are transferred to a fermentation chamber (25°C) where they remain for 20 min. Each disc of dough, in turn, is then transferred to the Alveograph where it is inflated and the curve recorded. At the moment the bubble ruptures, the volume of the liquid in the gasometer is recorded ("G" value). Three measurements are taken from the mean curve: maximum height (cm), which measures the pressure applied during inflation; maximum length (cm), which measures the extensibility of dough from first application of pressure to the point where the surface of the bubble ruptures; and area under the curve (in square cm). With the aid of the "G" value and the area, a value "W" is computed using the

<sup>3</sup> Since these studies were made, the tension on the balance has been changed to correspond to 200 paper units with a balancing weight of 470 g.

formula:  $W = K \times C \times S/L$ ; where  $K$  is a constant (manometer correction coefficient);  $C$ , a value associated with the volume of liquid in the gasometer;  $S$ , the area under the curve; and  $L$ , the length of the curve in millimeters. The value for  $W$  is considered to be a quantitative measure of the total work (number of ergs) required to extend the dough. The standard error of the mean of five replicate curves is of the order of  $\pm 0.4$  cm for an average length of 11 cm, and  $\pm 0.2$  cm for an average height of 8.5 cm.

*Baking.* All loaves were baked by the malt-phosphate-bromate procedure according to the method outlined by Geddes *et al* (1940).

### The Variable Protein Series

About 7,000 samples of wheat, of grades 1, 2, and 3 Northern, were collected and analyzed for a protein survey of the 1939 crop of Western Canadian wheat. These were subsequently composited within 0.5% protein ranges to provide a series of samples for the present investigation. A number of the composite samples, at the extreme ends of the protein range, were too small for studies with all machines, but eight samples ranging in protein content from 11.7 to 15.0% were obtained. Annual surveys have shown that in most years 70% of the Western Canadian wheat crop falls within this protein range.

The wheats were milled to yield in a Buhler mill and the resulting flours had the following protein contents and loaf volumes:

Protein content, %	11.1	11.4	12.1	12.4	12.9	13.6	14.1	14.4
Loaf volume, cc	600	635	680	715	765	750	845	875

In any series of composite samples, each representing several hundred cars of grain of essentially the same protein content, all variations in properties except those closely associated with protein content are eliminated. The series then serves admirably for investigations of relations with protein content, but for nothing else. For instance, it will not serve for a study of the relations between Farinogram measurements and loaf volume; for loaf volume is so closely related to protein content ( $r = 0.99$ ) that every property that is correlated with the latter must also be correlated with the former. Nor can this problem be solved by partial correlations; for the variations in loaf volume that are not associated with variations in protein are so small as to provide no scope for partial correlations. Accordingly, it will be profitable with the variable protein series to deal only with the relations between the curve measurements (for Farinograms, Extensograms, and Alveograms) on the one hand, and protein content on the other.

As the curves are more informative than tables of measurements of them, the curves are reproduced in Figure 2. The normal and high-

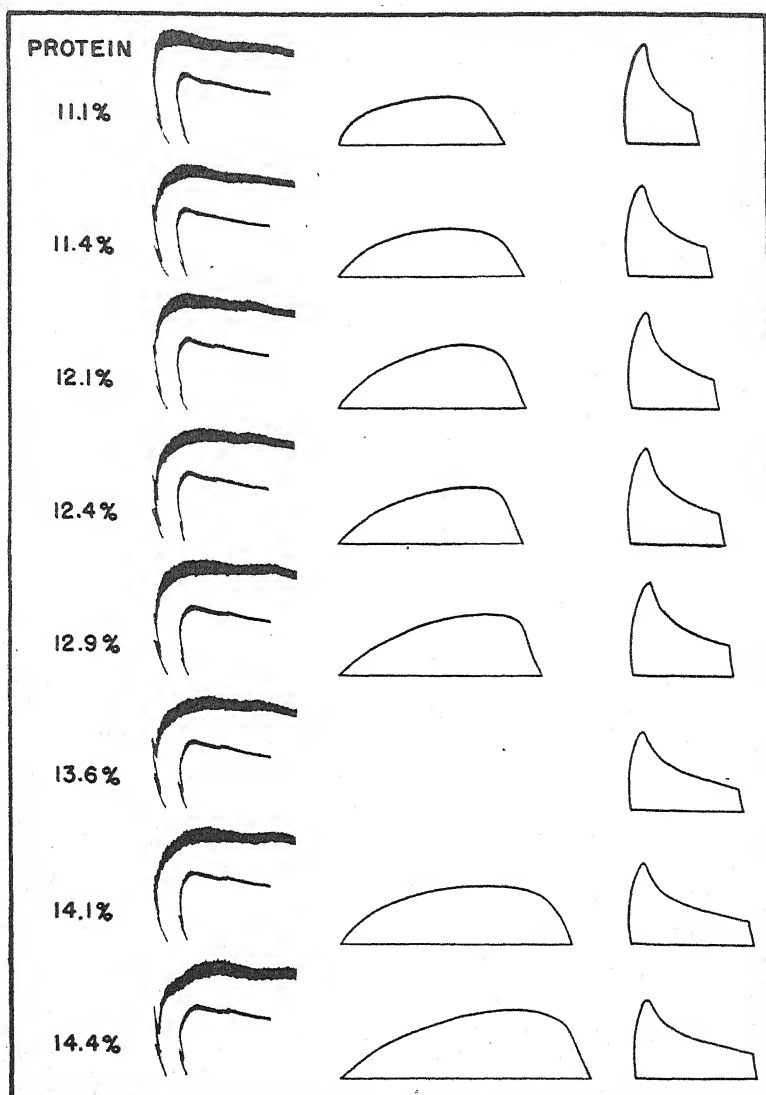


Fig. 2. Farinograms (left), Extensograms (center), and Alveograms (right) for composite samples of increasing protein content.

speed Farinograms are on the left; the Extensograms (135-min rest period) are in the middle; and the Alveograms are on the right.

*Farinograms.* As the protein increases from 11.1 to 14.4%, the following changes occur in the Farinograms: (1) the time (development time) required to produce maximum dough consistency increases from 2.75 to 7.0 min in the normal curves, and from 1.75 to 3.0 min in the

high-speed curves; (2) the rate at which the dough loses its maximum consistency decreases in both types of curve; and (3) the time (break time) required to bring about the change in consistency that occurs when the dough becomes sticky—shown by a break in the falling curve—increases from 4.0 to 5.75 min in the high-speed curves. In short, with increasing protein content there is a gradual change from a sharply rising curve, which falls off rapidly and shows an early break (more apparent in the high-speed curves), to a curve that rises more slowly and drops not at all or less rapidly.

The closeness of the associations between these changes and changing protein content can be summarized by correlation coefficients; but high correlations must be discounted because there are only eight pairs of values ( $n = 6$ ). The coefficients,<sup>4</sup> listed below, for all Farinogram measurements are all of essentially the same order; the regression coefficients, the average increase in each measurement per 1% increase in protein content, are also listed.

	Correlation coefficient	Regression coefficient
Normal, development time $\times$ protein	0.94**	1.13
High-speed, development time $\times$ protein	0.95**	0.36
High-speed, break time $\times$ protein	0.92**	0.58

These values are considerably lower than the correlation between protein content and loaf volume,  $r = 0.99$ . In general, the results are in accord with those reported by Geddes *et al* (1940) for development time (normal curve), and by Malloch (1938), who studied the break in curves produced by a recording mixer of his own design.

*Extensograms.* Figure 2 shows that with increasing protein content the length of the curve, which represents the distance that the dough stretches before it breaks, increases fairly regularly from 16.4 to 25.0 cm; and that the height of the curve, which measures the resistance of the dough to stretching, increases over a narrower range, and less regularly, from 4.6 to 6.6 cm. The curves become much longer and a little higher as protein content increases. Though the area under the curves was not measured, it is obvious from inspection, and from consideration of the data on length and height, that it increases with increasing protein content; the observation of Munz and Brabender (1940a) is thus confirmed.

There are only seven pairs of values for calculating correlation and regression coefficients and this point must be borne in mind in considering the following statistics.

	Correlation coefficient	Regression coefficient
Extensibility $\times$ protein	0.96**	2.26
Resistance to extension $\times$ protein	0.77*	0.46

<sup>4</sup> Throughout this paper, a single asterisk is used to denote that the correlation coefficients exceed the 5% level of significance, and double asterisks show it exceeds the 1% level.



The correlation for extensibility (length) and protein is of the same order as the Farinogram correlations, but that for resistance to extension (height) is considerably lower.

*Alveograms.* As protein content increases there is a regular increase in the length of the Alveogram from 7.2 to 12.4 cm, and a regular decrease in height from 10.0 to 7.9 cm. In addition, the work done (as calculated from the formula given previously) increases from 325 to 428 units. The length of the curve represents the time required to burst the bubble, which is inflated under an essentially constant head of water; length is thus a measure of the extensibility of the dough. The height of the Alveogram measures the maximum pressure under the dough just after inflation starts; it appears to be associated with the stiffness, shortness, and tightness of the dough. Thus Alveogram height represents an entirely different property from Extensogram height; indeed this is obvious from the curves, which show that the height of the Alveogram decreases and that of the Extensogram increases with increasing protein content. On the other hand, the lengths of both curves measure essentially the same property, extensibility, albeit under different conditions of extension. On the whole, it appears that in the Alveograms the work done ( $W$ ) provides the best measure of resistance to extension, the property represented by the height of the Extensogram. Both these measurements increase with increasing protein content.

The correlation and regression coefficients ( $n = 6$ ) for the Alveogram measurements and protein content are:

	Correlation coefficient	Regression coefficient
Extensibility (length) $\times$ protein	0.99**	1.54
Initial stiffness (height) $\times$ protein	-0.88**	-0.62
Resistance to extension ( $W$ ) $\times$ protein	0.74*	25.2

The extensibility, as measured by the Alveograph, is apparently more closely related to protein content than any other property measured by this or the other machines; the correlation coefficient is as high as that for protein and loaf volume, though it is by no means certain that this result would be confirmed by a more extensive study. The correlation for protein and resistance to extension is of the same order for the Alveogram as for the Extensogram. It will also be noted that the height of the Alveogram is the only measurement on all types of curves that is inversely related to protein content.

*Regression Coefficients.* For practical purposes, the regression coefficients that have been listed in the preceding subsections appear to represent the most useful information contained in this paper. Moreover, they should be quite reliable because, though only eight or nine

pairs of values were available for calculating them, the samples represent many subsamples composited to give a series that can vary only in protein content and in properties closely associated with it.

It will have been noted that the changes in the curve measurements for a 1% increase in protein content are quite large: the development time of the normal Farinogram increases by 1.1 min; the length and height of the Extensogram increase by 2.26 cm and 0.46 cm, respectively; Alveogram length increases by 1.54 cm, and height decreases by 0.62 cm. Under these conditions, it is clear that the interpretation of any of these curves for samples of different protein content (such as are found in any normal series of commercial samples) is likely to be based mainly on the effects of the differences in protein content, unless pains are taken to discount them. The regression coefficients are especially useful for this purpose.

### The Uniform Protein Series

The samples in this series represented individual parcels of grain shipped from different areas of Western Canada, but it is almost certain that each was a mixture of wheats from different farms. A number of samples of the same protein content were first collected. These were milled and the flours were baked, and the resulting data were used to select samples of flour of essentially uniform protein content giving loaves of about the same size. Nine samples were obtained, and the protein contents and loaf volumes for these were:

Sample	B	I	D	C	A	G	E	F	H
Protein content, %	13.1	13.2	13.2	13.2	13.3	13.3	13.4	13.4	13.5
Loaf volume, cc	745	785	740	750	790	740	755	795	745

The spreads were thus 0.4% for protein content and 55 cc for loaf volume. It should be added that no differences between the handling properties of the doughs could be detected by the baker.

This series makes it possible to examine (1) the ability of the machines to differentiate between samples that cannot be differentiated by the protein determination and the malt-phosphate-bromate baking test, and (2) to examine the relations between the measurements made by the different machines. It will be noted that the second study is equivalent to the calculation of partial correlation coefficients independent of both protein and loaf volume; and it is much more effective and convincing than the calculated partial correlation coefficients.

It was the intention to obtain a series of samples with equal protein content but different loaf volumes, and a series of samples with equal loaf volumes but different protein contents. Studies of these would have been equivalent to calculations of partial coefficients independent

of loaf volume, and of partial coefficients independent of protein content. Unfortunately, it was not possible to collect suitable series of these types.

*Differentiation between Samples.* The curves for this series, arranged in order of increasing Alveogram length, are shown in Figure 3. Though the spread in protein content was only 0.4%, there is about as much variation in curve type as in the variable protein series which has a spread of 3.3%.

In the normal Farinograms, development time ranges from 4.75 min for sample G to 8 min for sample C; but in the high-speed curves, the spread is only 2.25, *F, G, and H*, to 2.75, *A, B, C, D, and E*. On the other hand, the spread in the break time of the high-speed curves is comparatively large, from 5.25 min for *F* and *I* to 8.0 min for *C*.

Among the Extensograms, the extensibility (length) varies from 19.8 cm for *F* to 23.8 cm for *D*; and the resistance to extension (height) varies from 5.3 cm for *I* to 8.9 cm for *C*.

The Alveograms are no more uniform. Extensibility (length) ranges from 9.7 cm for *A* to 12.6 cm for *I*; stiffness (height) from 7.8 cm for *G, H, and I* to 10.6 cm for *A*; and work done (*W*) from 390 units for *F* to 504 units for *D*.

It is thus apparent that all machines were able to demonstrate that differences in physical properties existed between samples that differed little in protein content or in loaf volume obtained with the malt-phosphate-bromate formula.

The machines do not agree entirely on which samples are similar and which dissimilar. That the Farinograph should yield results that differ from those of the other machines is hardly surprising since it operates on an entirely different principle and measures different properties. In development time, the high-speed and normal curves show moderate agreement—possibly as much as could be expected with such little differentiation between high-speed curves. The three samples that have normal curves with the shortest development times are classified as equal and lowest (2.25 min) by the high-speed curves; the four samples that have normal curves with the longest times are classified as equal and high (2.75 min) by the high-speed curves; and of the remaining two curves, which have values of 5.5 min for the normal curves, one is classified as high (2.75 min) and one as intermediate (2.5 min) by the high-speed curves.

The Extensograms and Alveograms might reasonably be expected to classify samples in the same way because they measure similar properties. They did not invariably do so. For instance, on the basis of dough extensibility (length) both machines classify samples *A, B, C, E, and H*, in the same manner; but the Extensogram classifies

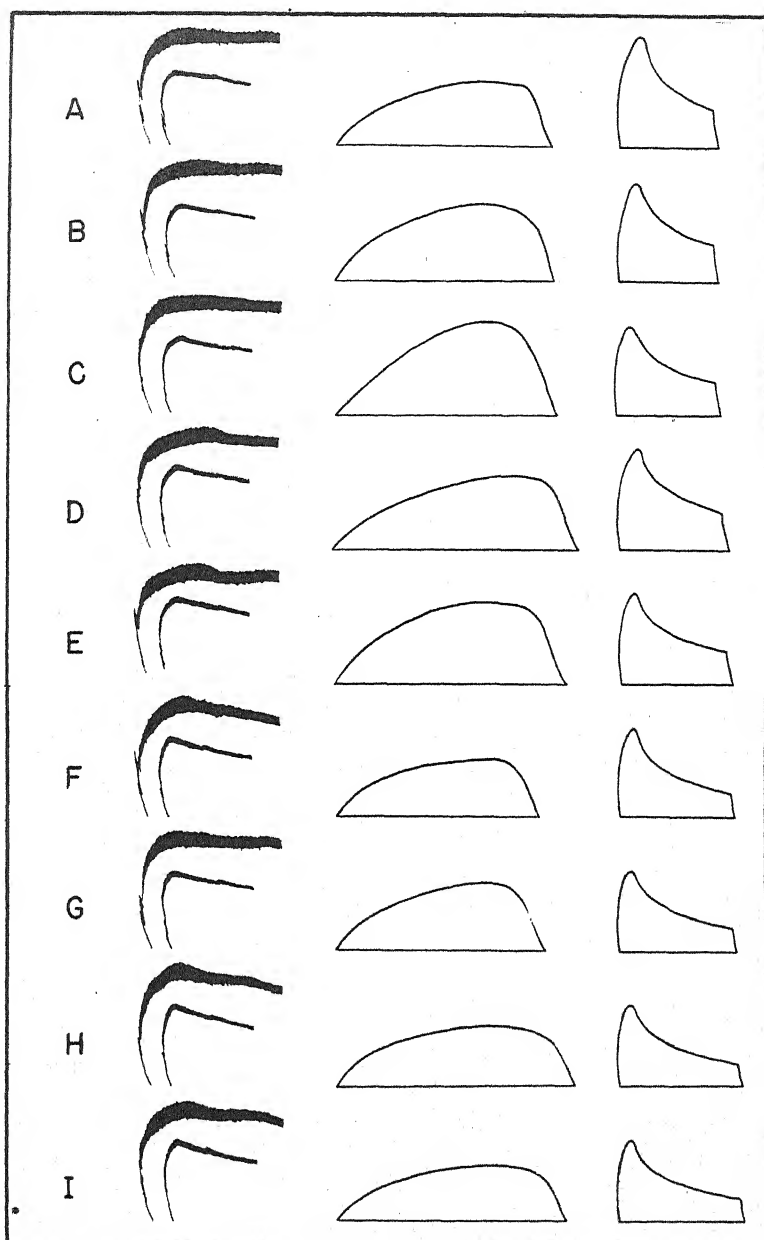


Fig. 3. Farinograms (left), Extensograms (center), and Alveograms (right) for individual samples of essentially the same protein content.

sample *D* as highly extensible and samples *F* and *G* as having little extensibility, whereas the Alveograph classified all three samples in the intermediate range. In addition, sample *I*, which is most extensible according to the Alveograph, is only in the middle range for the Extensograph.

If the height of the Extensogram and the work done (*W*) by the Alveograph can be considered to measure essentially the same property, resistance to extension, then a further comparison can be made. The machines agree that samples *F*, *H*, and *I* have low values (Alveogram *W* = 390, 395, and 397 units; Extensogram height = 5.5, 5.8, and 5.3 cm), and that samples *B* and *E* have fairly high values (Alveogram *W* = 441 and 447; Extensogram height = 7.4 and 7.9 cm). But sample *C* is classed as highest by the Extensograph (8.9 cm) and only as intermediate by the Alveograph (*W* = 428), whereas samples *D* and *A* (*W* = 504 and 482) are classed as highest by the Alveograph and only as intermediate by the Extensograph (height = 7.1 and 6.4 cm).

*Relations among Curve Measurements.* The relations between the various pairs of curve measurements can best be studied with the aid of the correlation coefficients given in Table I. Those for development

TABLE I  
CORRELATION COEFFICIENTS FOR CURVE MEASUREMENTS  
Nine samples (*n* = 7)

Variable	Farino- gram, high-speed break time	Extensogram		Alveogram		
		Height	Length	Height	Length	Work
<i>Farinogram</i>						
Normal development time	0.72*	0.80**	0.40	0.34	-0.44	0.59
High-speed break time		0.95**	0.11	0.23	-0.50	0.40
<i>Extensogram</i>						
Height			0.15	0.27	-0.58	0.43
Length				0.07	0.19	0.44
<i>Alveogram</i>						
Height					-0.84	0.88**
Length						-0.69*

time of the high-speed Farinograms have not been included because the machine classified all samples but two into two distinct groups, and there is thus the danger of obtaining significant correlation coefficients which are entirely spurious. All other measurements cover their ranges with sufficient uniformity to avoid this pitfall.

The highest correlation is between high-speed break time and the height of the Extensogram. A fundamental relation may well be involved, because break time represents the amount of mixing the dough will stand before it "breaks down" and becomes sticky, and

the height of the Extensogram represents the distance that the dough can be stretched before it breaks; it is thus conceivable that both measurements are related to essentially the same dough property. Both break time and Extensogram height are also related to the development time of the normal Farinogram, *i.e.*, to the amount of mixing required to develop maximum consistency of the dough. It thus appears that all three measurements are controlled, in part, by the same dough property or properties.

The only other significant correlation coefficients involve Alveogram measurements. It is hardly surprising that the work (*W*) is related to both height and length of the curve because *W* is a function of the area under the curve, and height and length are involved in this measurement. There is also a high correlation between Alveogram height and Alveogram length, and this is inverse—the greater the height, the less the length. If it is correct that height measures mainly the stiffness and shortness of the dough, and that length measures mainly its extensibility, then the inverse correlation seems reasonable; short, stiff doughs are least extensible. In considering Table I the reader should bear in mind that there were only nine pairs of values for each correlation, and it is therefore quite probable that a larger study would demonstrate additional significant correlations.

There is one other matter of special interest: not only is there no significant correlation between Alveogram length and Extensogram length but the correlation coefficient is the fourth lowest of those listed. This seems surprising since both measurements might reasonably be expected to reflect the extensibility of the dough. It must therefore be assumed that the measurements are made under such widely differing conditions that no correlation between the results is possible.

Several differences between the techniques used with the two machines are readily apparent. First, Extensograms are made with doughs of uniform consistency whereas Alveograms are made at uniform absorption. If the former technique is the better for obtaining a "true" measure of extensibility, then it is easy to understand that the latter technique may give contradictory results with certain samples, and *vice versa*. Second, the Extensograph stretches the dough in only one direction, whereas the Alveograph stretches it in all directions. Third, the Extensograph stretches the dough at a constant rate, which is essentially independent of the resistance of the dough to stretching; whereas the rate at which the Alveograph bubble expands and stretches the dough must be affected by the resistance of the dough to stretching. And fourth, whereas the Extensograph dough is made with water and sufficient salt solution to bring the salt content to 2%, the Alveograph dough is made with 2.5% salt solution and is also

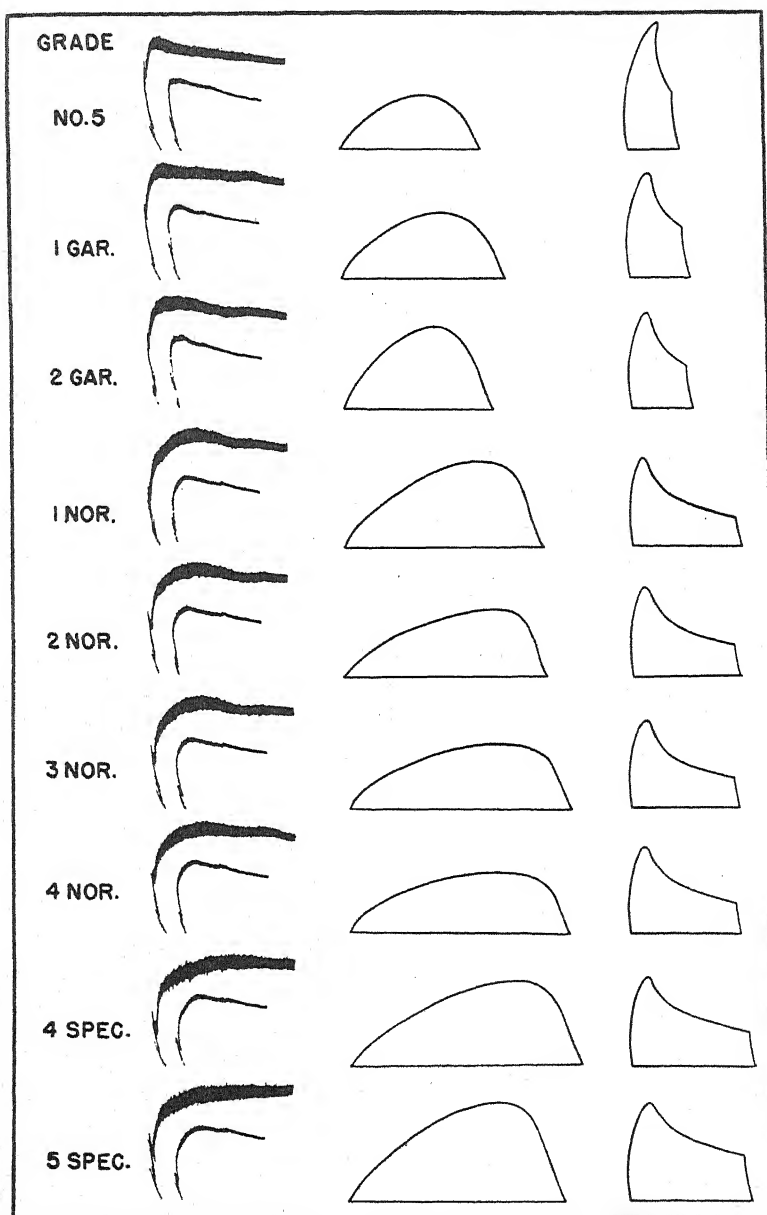


Fig. 4. Farinograms (left), Extensograms (center), and Alveograms (right) for average samples of the grades of the 1939 crop of Western Canadian wheat.

lubricated with oil. In these circumstances perhaps it is too much to expect the two machines to place a series of dough samples in essentially the same rank order with respect to extensibility. Further investigation of this matter is obviously required.

### Grade Series for 1939 Crop

Composite samples representing all cars of grain inspected in the Western Division during the 1939-40 crop year were prepared for the grades 1 to 4 Northern, 4 and 5 Special, No. 5, and 1 and 2 C.W. Garnet. Similar samples are prepared each year for certain routine studies, but as 1939-40 was the last crop year in which all these grades were represented, it is fortunate that this investigation was undertaken at that time. The introduction of rust-resistant varieties has made it unlikely that wheat will be graded into the Special grades in future unless there is a severe and widespread drouth. Moreover, during the past few years, the production of Garnet wheat has dropped rapidly and it seems probable that the variety and its grades will disappear entirely within a year or two.

Each of the composite samples represented several million, and some represented many million bushels of wheat. Moreover, the 1939 crop, with a mean protein content of 14.1%, was not far from the long-time average for Western Canadian hard red spring wheats.

*Differences among Grades.* The curves for the grade series are shown in Figure 4, and the data for protein content, loaf volume, and curve measurements are given in Table II. In both the figure and the table,

TABLE II  
PROTEIN CONTENT, LOAF VOLUME, AND CURVE MEASUREMENTS  
FOR GRADE SERIES, 1939 CROP

Grade	Protein content	Loaf volume	Farinogram			Extensogram		Alveogram		
			Normal	High-speed		Length	Height	Length	Height	Work
				Dev. time	Break time					
	%	cc	min	min	min	cm	cm	cm	cm	units
No. 5	11.1	460	2.0	1.5	5.0	14.1	5.2	5.3	12.6	333
1 Gar.	11.5	525	2.5	2.0	4.5	14.9	8.0	6.0	10.3	320
2 Gar.	11.5	525	3.0	2.0	4.5	16.3	6.4	6.2	9.4	289
1 Nor.	13.1	780	5.5	2.5	5.75	19.8	8.4	11.0	8.3	387
2 Nor.	13.0	765	5.5	2.5	6.0	20.5	6.7	10.9	8.8	410
3 Nor.	13.4	785	5.5	2.5	5.75	22.0	6.4	11.8	8.8	400
4 Nor.	13.6	750	6.0	2.75	5.25	22.1	6.0	11.1	8.7	422
4 Spec.	15.8	885	8.75	3.5	6.0	23.2	8.3	12.3	9.0	499
5 Spec.	16.5	920	10.0	3.5	6.0	21.5	9.7	12.1	10.0	557



the grades are arranged in order of increasing protein content, except that 1 Northern with a protein content of 13.1% has been placed above 2 Northern, which has a protein content of 13.0%.

Figure 4 shows that the curves divide the grades into three groups: No. 5 and the two Garnet grades, the four Northern grades, and the two Special grades. The Farinograms for the first group have short development times and sharp peaks, those for the second group have intermediate development times and rounded peaks, and those for the last group have very long development times and no pronounced peaks. The Extensograms and Alveograms for the first group are short, those for the middle group are intermediate, and those for the last group are long; and the Alveograms for the first and last groups are higher than those for the middle group.

Both protein content and loaf volume classify the samples into exactly the same groups. The values are low for the first group, intermediate for the middle group, and high for the last group. It is thus clear that a large proportion of the differences in curve measurements between grades is caused by differences in protein content. Accordingly, with this series, it will be most profitable to examine each group of grades separately to determine whether grading factors have an effect on curve measurements that is independent of the effect of protein content.

The two Garnet grades have the same protein contents and might be expected to give identical curves. The Farinograms are much the same, except that the normal curve for 2 C.W. Garnet has a slightly longer development time than that for 1 C.W. Garnet. There is a greater difference between the Extensograms; that for 2 C.W. Garnet is shorter and taller than that for 1 C.W. Garnet. Between the Alveograms, there is little difference in length, but that for 1 C.W. Garnet is higher and gives a higher W value than that for 2 C.W. Garnet.

The Northern grades show little difference in the development times of the Farinograms. But, neglecting 1 Northern, there is evidence of a slight decrease in break time with decreasing grade; and this is contrary to expectations based on protein content, which increases with grade. In accordance with the results obtained with the variable protein series, Extensogram height and length would be expected to increase with increasing protein content, *i.e.*, to increase slightly with decreasing grade. The length increases about as much as would be expected, but in 1 to 4 Northern, grading factors reverse the trend for height, which decreases with grade. Among the Alveograms, there are only small differences in length, somewhat larger ones in work, and no evidence of the decreases in height which should occur with increasing protein content. On the whole, the curves for different grades are

very similar. The small differences in protein content are not reflected, but neither is there any clear cut effect of grade.

Although there is a relatively large difference in protein content between the special grades, this is not reflected in all curve measurements. For example, the high-speed Farinograms are identical though there is an increase in the development time of the normal curve. No. 5 Special has a shorter Extensograph length and a greater Alveogram height than No. 4 Special; and these results are contrary to expectations based on protein content.

The 1939 grade series therefore shows that though protein content has a very large effect on curve type, there may be other dough properties, related to grading factors, that affect the curves and tend to upset expectations based on protein content alone. Additional information on this point can be obtained by means of the correlation coefficients given in the following subsection.

*Correlation Studies.* The pertinent correlation coefficients for the grade series are listed in Table III. Coefficients for the simple correla-

TABLE III  
CORRELATION COEFFICIENTS FOR CURVE MEASUREMENTS,  
PROTEIN CONTENT, AND LOAF VOLUME

Nine pairs of values ( $n = 7$ ) for simple correlations, and  $n = 6$  for partial correlations

Curve measurements	Simple correlations		Partial correlation <sup>1</sup> With loaf volume
	With protein content	With loaf volume	
<i>Farinogram</i>			
Normal, development time	0.99**	0.95**	0.71*
High-speed, break time	0.78*	0.89**	0.23
<i>Extensogram</i>			
Length	0.83**	0.94**	0.80*
Height	0.68*	0.61	-0.08
<i>Alveogram</i>			
Length	0.87**	0.98**	0.96**
Height	-0.38	-0.68*	-0.98**
Work (W)	0.98**	0.91**	-0.01

<sup>1</sup> Independent of protein content.

tions between curve measurements and protein content are given in the first column of data. Except for that with Alveogram height, all coefficients are significant, though two attain only the 5% level. The high correlations must be partially discounted because there are only nine pairs of values, but it is apparent that most of the differences between grades with respect to dough development time and Alveogram work are associated with differences in protein content. With respect to other measurements, there is evidence of variations that are independent of protein content.

The simple correlations between curve measurements and loaf volume are very interesting. Some are lower than the corresponding correlations with protein content, and some are higher. The latter are the more interesting because they suggest a relation between the curve measurement and loaf volume that is independent of the relation between loaf volume and protein content. The measurements in question are break time, Extensogram length, and length and height of the Alveogram; all these are more closely correlated with loaf volume than with protein content.

In order to examine these relations further it is necessary to calculate the partial correlations between curve measurements and loaf volume, independent of protein content. This procedure gives approximately the same results as would be obtained from a series of samples of the same protein content that differed in curve measurements and loaf volumes. The partial correlation coefficients exceed the 1% level of significance for Alveogram length and height, and the 5% level for development time of the normal Farinogram and for Extensogram length.

Accordingly, these measurements tell something about the loaf volumes that the sample will give that is not told by protein content alone. Among flours of equal protein content, those having longer development times may be expected to give the larger loaves by the malt-phosphate-bromate procedure; those having the greater extensibility, as measured by the length of either the Extensogram or the Alveogram, will give larger loaves; and those that are least stiff (*i.e.*, that give low Alveograms) will give the larger loaves. These results, especially those relating to Alveograms, appear to be quite definite in spite of the small number of samples available for the study.

As a matter of interest, the multiple correlation coefficient for loaf volume on the one hand, and protein content and Alveogram height on the other, was calculated. This turned out to be  $R = 0.998^{**}$  and this is significantly higher (1% level) than the corresponding simple correlation between protein content and loaf volume,  $r = 0.930^{**}$ . Corresponding coefficients for the variable protein curves were  $R = 0.997^{**}$  and  $r = 0.992^{**}$ ; and the significance of the added variable exceeded the 5% level. These statistics suggest that it may well be possible to predict loaf volumes more accurately from protein content and Alveogram height than from protein content alone. But, as baking tests can be made more readily than Alveograms, further investigation of this matter with a larger series of samples, though it may be of some theoretical interest, holds little promise of elaborating practical procedures.

### Northern Grades Series

The samples in this series represented grades 1, 2, 3, and 4 Northern for each of the five years 1939 to 1943. For 1939, the samples represented all shipments made during the 1939-40 crop year. For the remaining years, the sample for each grade was made up by compositing several hundred individual samples collected all over Western Canada during the first eight or nine weeks after the crop started to move. The composite samples were used to obtain preliminary information on the quality of each new crop, and as the Farinograms, Extensograms, and Alveograms have been reproduced in the annual crop bulletins issued by the laboratory, there is no need to present them again. Data on protein content, loaf volume, and curve dimensions for all samples are given in Table IV.

The data have been exhaustively and repeatedly studied by the authors, both individually and collectively, but to little avail. All conclusions are essentially negative.

No consistent trends can be discovered by considering the differences between grades within years. Because of the relatively small differences in protein content between grades, and keeping in mind the experimental errors, one would hardly expect to discover consistent relations between protein content and curve measurements in these sets of samples. Some effect of grade on curves might be expected if dough properties reflect any of the major grading factors. So far as the authors can determine, there is no evidence among the data for the individual years of a consistent relation between grade and any of the curve dimensions. And this conclusion is certainly supported by the mean data for each grade over all years; for the differences between grades are essentially negligible and by no means consistent.

If the lengths for both Extensograms and Alveograms measure the extensibility of the dough, then in spite of some general agreement there is ample evidence of the ability of the two machines to contradict each other. For instance, in 1940 the Extensograms indicate that 3 Northern is decidedly more extensible than 4 Northern, but the Alveogram indicates that 4 Northern is slightly more extensible than 3 Northern. Again, in 1939 for grades 1 and 4 Northern, and in 1941 for grades 2 and 3 Northern, the Alveograph finds similar extensibilities while the Extensograph finds appreciable differences. And again, in 1942, the Extensograms indicate similar extensibilities for grades 3 and 4 Northern, but the Alveograms suggest a considerable difference between the grades. Until such discrepancies are explained by further investigation, little faith can be placed in data obtained with either machine unless there is some *a priori* reason, of which the authors are not aware, for preferring one to the other.

TABLE IV  
PROTEIN CONTENT, LOAF VOLUME, AND CURVE MEASUREMENTS FOR  
GRADES 1, 2, 3, AND 4 NORTHERN

Year	Grade	Flour protein content	Loaf volume	Farino- gram develop- ment time	Extensogram 135" curve		Alveogram		
					Length	Height	Length	Height	"W"
		%	cc	min	cm	cm	cm	cm	units
1939	1 Nor.	13.1	780	5.5	19.8	8.4	11.0	8.3	387
	2 Nor.	13.0	765	5.5	20.5	6.7	10.9	8.8	410
	3 Nor.	13.4	785	5.5	22.0	6.4	10.8	8.8	400
	4 Nor.	13.6	750	6.0	22.1	6.0	11.1	8.7	422
1940	1 Nor.	13.4	785	5.0	18.7	5.0	9.0	9.5	353
	2 Nor.	13.6	780	5.5	19.3	4.8	9.6	9.7	385
	3 Nor.	13.9	820	5.25	19.2	4.5	11.0	8.5	312
	4 Nor.	13.6	750	5.0	13.5	6.4	11.9	8.3	289
1941	1 Nor.	13.8	815	6.5	20.7	7.4	10.5	8.6	448
	2 Nor.	14.2	845	7.0	19.6	7.4	11.5	9.0	494
	3 Nor.	14.1	840	7.0	22.5	7.8	11.2	9.0	507
	4 Nor.	14.4	900	6.0	24.5	7.8	12.3	8.0	491
1942	1 Nor.	12.1	710	5.5	17.4	7.6	8.1	9.2	383
	2 Nor.	12.0	705	4.5	17.0	7.2	9.4	9.3	417
	3 Nor.	12.1	740	5.5	15.6	6.8	9.4	9.2	429
	4 Nor.	12.0	720	5.0	15.6	6.5	7.6	9.8	392
1943	1 Nor.	13.3	795	4.5	19.4	5.4	11.5	7.7	383
	2 Nor.	13.1	795	4.5	19.4	5.0	11.1	7.7	415
	3 Nor.	12.6	775	4.0	18.8	4.2	9.8	7.9	349
	4 Nor.	12.6	770	3.5	17.5	3.5	8.3	8.5	339
All years	1 Nor.	13.1	775	5.5	19.2	6.8	10.0	8.7	391
	2 Nor.	13.2	780	5.5	19.2	6.2	10.5	8.9	424
	3 Nor.	13.2	790	5.5	19.6	5.9	10.4	8.7	399
	4 Nor.	13.2	780	5.0	18.6	6.0	10.2	8.7	387
1941	All grades	14.1	850	6.5	21.8	7.6	11.4	8.6	485
1940		13.6	785	5.25	17.7	5.2	10.4	9.0	335
1939		13.3	770	5.5	21.1	6.9	11.0	8.6	405
1943		12.9	785	4.0	18.8	4.5	10.2	8.0	372
1942		12.0	720	5.0	16.4	7.0	8.6	9.4	405

When the average differences between crops are considered with the aid of the means for all grades, some general trends can be discerned. Part, but by no means all, of the differences in curve measurements between crops can be traced to differences in protein content. Moreover, when the five crops are placed in order with respect to protein content, the 1940 crop is consistently out of order, and in the right direction, for all curve measurements. In addition, the 1943 crop, which is out of order for development time, is also out of order for Extensogram height and Alveogram "W," and for Alveogram height, though in the wrong direction. There is also some agreement between extensibility measurements by both the Extensograph and the Alveo-

graph; both machines place 1941 first, 1939 second, and 1942 last with respect to length. But these are the three crops on which all machines and measurements agree in placing the crops in order of their protein contents. Thus, on the whole, the mean values for each crop are not particularly illuminating.

The series under discussion certainly fails to provide any evidence that grade and factors associated with it bear any relation to the dough properties measured by the machines. This, of course, is no disparagement; for protein content and loaf volume, both of which are useful criteria of wheat quality, are not related to grade either.

### Summary

Four series of samples of Western Canadian wheat were employed in a study of the effect of protein content and grade on Farinograms, Extensograms, and Alveograms. Farinograms made with a high speed mixer (2.3 times normal speed) were also studied. These provided an additional measure, break time; this represents the time that elapses before the dough "breaks down" and becomes sticky, which causes a sharp break in the Farinogram.

The first series consisted of eight composite samples ranging in protein content (flour) from 11.4 to 14.4%. All curve dimensions were directly correlated with protein, except Alveogram height with which the correlation was inverse. The regression coefficients of the principal curve dimensions on protein content were: dough-development time of Farinogram 1.1 min; Extensogram length and height, 2.26 and 0.46 cm; and Alveogram length and height, 1.54 and  $-0.62$  cm, per 1% increase in protein content.

The second series consisted of nine individual samples of essentially the same protein content and baking strength. All machines demonstrated that differences in dough properties existed between the samples; but even the Extensograph and Alveograph, which appear to measure much the same dough properties, did not always agree on which samples were similar and which dissimilar. Moreover, no correlation was apparent between Extensogram length and Alveogram length, both of which measure the extensibility of the dough—though under different conditions. The highest correlation was obtained between break time and Extensogram height ( $r = 0.95$ ). Extensogram height was also correlated with development time of the normal Farinogram ( $r = 0.80$ ), and development time was correlated with break time ( $r = 0.72$ ). No other correlations between dimensions of curves from different machines were significant.

The third series consisted of composite samples of nine grades of the 1939 crop. All machines divided them into three groups: No. 5

and 1 and 2 C.W. Garnet; 1, 2, 3, and 4 Northern; and 4 and 5 Special. But determinations of protein content and baking strength (malt-phosphate-bromate) divided the series into exactly the same groups; group 1 was low in protein content and loaf volume, group 2 was intermediate, and group 3 was high. Within each group, there were slight indications that some dough properties were related to grading factors and this had a tendency to upset expectations based on protein content alone. Several curve dimensions were closely correlated with both protein content and loaf volume, and the partial correlations for Alveogram length ( $r = 0.96$ ) and for Alveogram height ( $r = 0.98$ ) and loaf volume, independent of protein content, exceeded the 1% level of significance.

The fourth series consisted of average samples of grades 1, 2, 3, and 4 Northern for the five years 1939 to 1943. The data for this series were anomalous, and certainly failed to demonstrate that any of the curve dimensions tend to change uniformly with decreasing grade.

#### Acknowledgments

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# REPRODUCIBILITY STUDIES AND SOME EFFECTS OF TECHNIQUE ON EXTENSOGRAMS AND ALVEOGRAMS <sup>1</sup>

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In a previous paper, Aitken, Fisher, and Anderson (1944) described the effects of the protein content and grade of wheat on Farinograms, Extensograms, and Alveograms. Before undertaking further work of that sort, it seemed desirable to make a more comprehensive study of the reproducibility of the curves, and to examine the effects of certain adjustments of the Extensograph, and of a variation in the procedure used with the Alveograph. The same flours were used with each machine, but in other respects the investigations were quite separate. Accordingly, this paper is divided into two sections dealing respectively with the Extensograph studies and the Alveograph studies.

## Extensograph Studies

With the Extensograph, the resistance of the dough to extension is measured by the pull that the dough exerts on one arm of a balance. The downward movement of the balance arm measures the force applied by the dough. As the movement is quite small, it is converted by a system of levers into a much larger movement of a pointer, which is equipped with a pen that writes on a kymograph. The increasing resistance of the dough to extension as it is stretched is thus recorded as increasing height of the Extensogram.

Weak and strong flours have widely different resistances to extension, and the lever system on the Extensograph is therefore made adjustable so that the machine can be used with both types of flour. At a suitable setting of the lever system, the pen responds readily to the comparatively small pulls exerted by weak flours, but if strong flours are used with this setting the curves rise rapidly and run off the kymograph paper. By adjusting the lever system to make the pen less responsive, curves for strong flours can be kept on the paper but those for weak flours hardly rise at all.

At one time or another, three different settings have been used in this laboratory, and it seemed desirable to obtain information about the relations between the curves obtained at these settings, and about the

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effects of setting on the sensitivity of the machine and reproducibility of its curves. One of the settings is widely used in Europe, and the second and third are both used on this continent.

*Specification for Settings.* The settings are specified by the movement of the kymograph pen for given loads on the dough holder. As the arm which supports the dough holder is part of the lever system, the load must be suspended at the same distance from the fulcrum as is the center of gravity of the dough when under test. The movements of the pen are recorded in the units represented by the lines on the kymograph paper, or in centimeters, which is the practice in this laboratory. By careful adjustment of the lever system a straight-line relation can be obtained between the load and the position of the pen on the paper. This point is illustrated by Figure 1, which shows the rela-

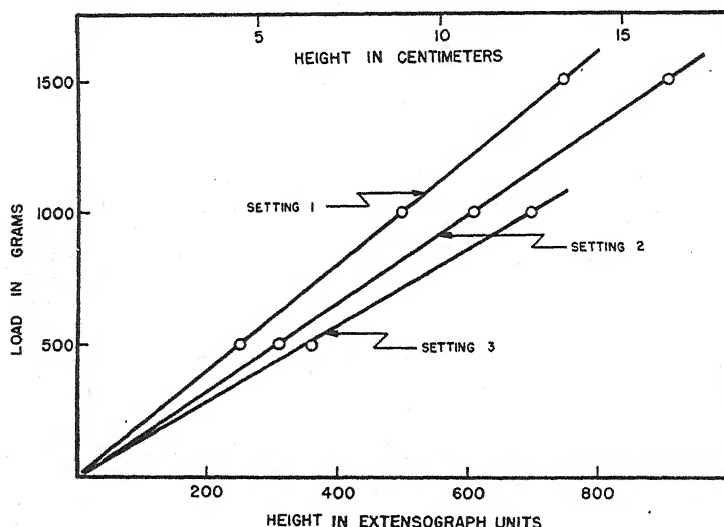


Fig. 1. Curves showing the relation between the load on the dough balance of the Extensograph and the height registered by the pen for three settings of the machine.

tions between load and pen for the three settings which were investigated.

It follows that laboratories using different settings of their Extensographs should be able to report resistance to extensibility (height of curves) on the same scale by converting the units on the kymograph paper to grams by means of an appropriate factor. Alternately, the height can be recorded in centimeters and a factor for converting this to grams can be computed. The factors for the three settings shown in

Figure 1 are 2.00, 1.63, and 1.41 for kymograph units, and 112.0, 91.5, and 79.0 for centimeters.

*Plan of Investigation.* The three flours chosen for this study were experimentally milled from (A) a 1 Northern wheat, (B) a No. 5 wheat, and (C) a 1 C.W. Garnet wheat. They had protein contents of 12.8, 13.5, and 11.1%.

The general technique employed with the Extensograph in this laboratory has been described by Aitken, Fisher, and Anderson (1944). The curves discussed in the present paper were made after a 45-min and 135-min rest period.

For each flour, duplicate 45-min and duplicate 135-min curves were made from the same doughs, in the morning and in the afternoon, on each of four different days during which one setting was maintained. This procedure was then repeated with each of the other settings. A factorial design was thus used, with three flours, three settings, eight replications (mornings and afternoons of four days), and duplicate curves.

*Results and Discussion.* The effects of the different settings on the shapes of the curves are of first interest. Figure 2 shows average

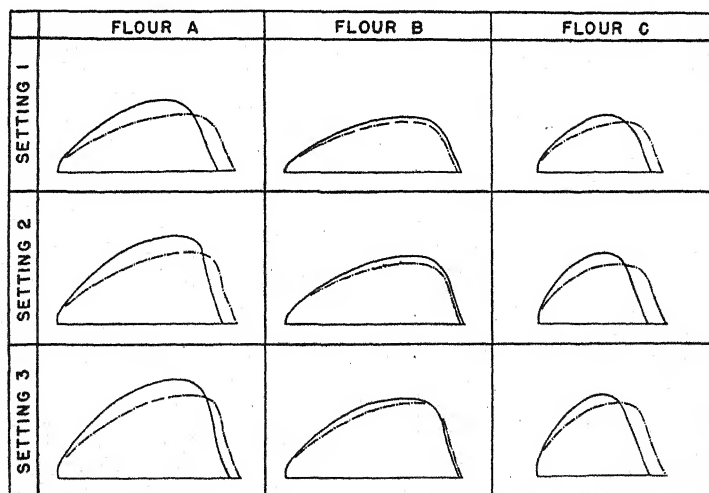


Fig. 2. Extensograms (45 min, dotted; 135 min, solid) for three settings of the machine.

curves for each of the three flours at each of the three settings, and data on length and maximum height are given in Table I. Inspection of the data indicates that the changes in the relative heights and lengths of the curves at different settings are essentially negligible, and statistical

analyses confirmed this conclusion. With settings representing increasing sensitivity, the length of the curve (extensibility of the dough) is not affected and the height of the curve increases. If the latter measurement, which represents resistance to extension, is translated into grams, most of the variation is removed. For each flour the data for all three settings agree within about  $\pm 2\%$ . Better agreement might possibly be obtained by more precise adjustment and calibration of the machine at each setting. However, it is clear that if different laboratories, using different settings for their Extensographs, would report resistance to extension in grams rather than in kymograph units or centimeters, the results could be more readily compared.

TABLE I

CURVE MEASUREMENTS FOR THREE FLOURS AT THREE EXTENSOGRAPH SETTINGS

Flour	Setting			Height			
	No.	Length		As measured		Converted to grams	
		45-min	135-min	45-min	135-min	45-min	135-min
A	1	cm 20.5	cm 18.5	cm 6.6	cm 8.1	g 740	g 910
	2	20.5	19.0	8.1	10.1	740	920
	3	20.7	19.5	9.8	11.3	770	890
B	1	20.0	20.4	5.7	6.3	640	710
	2	20.4	20.5	7.2	8.0	660	730
	3	20.5	20.1	8.7	9.2	690	730
C	1	14.4	13.1	5.7	6.5	640	730
	2	14.9	12.8	7.1	8.4	650	770
	3	14.9	13.0	8.5	9.6	670	760
Mean, all flours	1	18.3	17.4	6.0	7.0	670	780
	2	18.6	17.4	7.4	8.8	680	810
	3	18.7	17.6	9.0	10.0	710	790

The question of whether the flours were placed in exactly the same relative positions by the 45-min and the 135-min curves can be examined with the aid of Table II. There was no change in the length for flour *B* between the 45-min and 135-min curves, but lengths for flours *A* and *C* shortened appreciably; moreover, the order for flours *A* and *B* was reversed from one curve to the other. Again, for height as measured or converted, the order for flours *B* and *C* was reversed. It is thus clear that the rank order for certain flours will be affected by the length of the rest period between mixing and stretching. This is an interesting point because there appears to be no *a priori* reason for

TABLE II  
MEAN VALUES FOR EACH FLOUR OVER ALL SETTINGS FOR 45-MIN  
AND 135-MIN CURVES

Flour	Length		Height			
			As measured		Converted to grams	
	45-min	135-min	45-min	135-min	45-min	135-min
	<i>cm</i>	<i>cm</i>	<i>cm</i>	<i>cm</i>	<i>g</i>	<i>g</i>
A	20.6	19.0	8.2	9.8	750	910
B	20.3	20.3	7.2	7.8	660	720
C	14.7	13.0	7.1	8.2	650	750

selecting one rest period rather than another. Whether it is worth while as a routine procedure to use two rest periods and examine the changes that occur from one to the other with different flours has not yet been established.

The differences that can occur between curves made from the same flour by a standard method are illustrated in Figure 3. This shows

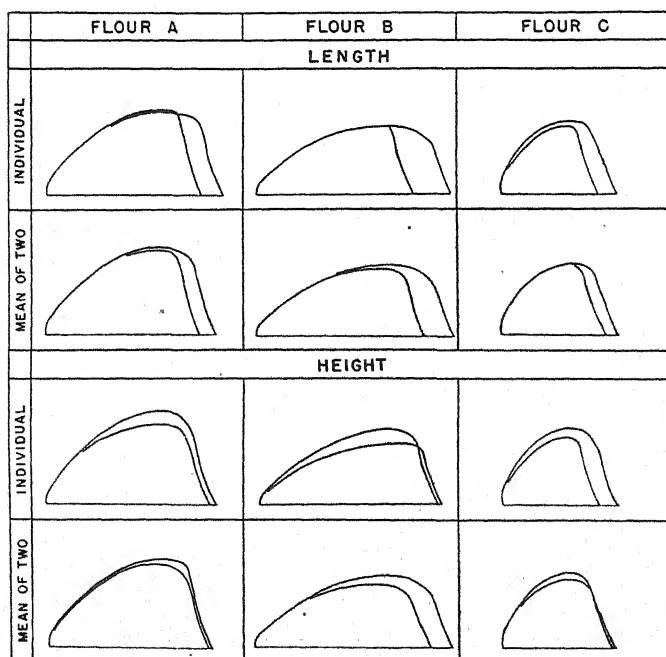


Fig. 3. Extensograms (135 min) showing the range in length and height obtained with individual curves, and with the mean curves (mean of 2 curves from one dough) for eight replicate doughs.

135-min curves for setting 2. Sixteen curves (2 for each of eight doughs) were made for flour *A*, and of these the longest and shortest are shown in the top left-hand corner of Figure 3. A similar selection from the eight curves representing means of two curves made from the same dough are shown immediately below. Corresponding pairs of curves for flours *B* and *C*, and the corresponding sets of curves for height, make up the rest of the figure. In drawing conclusions about the reproducibility of Extensograms, from this figure, the reader should bear in mind that the curves represent the maximum spreads obtained in the investigation.

Statistical analyses of the curve measurements showed that the differences between curves for doughs mixed on different days were not significantly greater than those between doughs mixed on the same day; all eight doughs from one flour can therefore be treated as samples from one population. Comparison of the inter-dough and intra-dough errors showed that the former were significantly greater for height at settings 1 and 3, and for length at setting 2. Although these results are not particularly clear cut, they indicate that in comparing two flours a better estimate of error will be obtained if single curves are made for duplicate doughs from each flour, in preference to duplicate curves for a single dough.

Data for the standard deviations for single curves, and for the mean of two curves made with duplicate doughs, are listed in Table III. These statistics are self-explanatory and require no comment.

TABLE III  
STANDARD DEVIATIONS FOR EXTENSOGRAM MEASUREMENTS

Measurement	Setting	Single curve	Mean of two curves from different doughs
Length, cm	1	0.92	0.65
	2	0.98	0.69
	3	0.93	0.66
Height, cm	1	0.73	0.52
	2	0.36	0.25
	3	0.68	0.48
Height, converted to g	1	82	58
	2	33	23
	3	54	38

### Alveograph Studies

The dough for the Alveograph is prepared in a special mixer, the Petrin. After mixing for 6 min, a slit in the side of the Petrin is opened by raising a slide. The continued operation of the mixer arm

then forces the dough out through the slit in a strip about 5 cm wide by 0.5 cm thick. As the strip is extruded, 5 to 6 cm lengths are cut off; these are rolled to standard thickness, and a disc (diam., 4.5 cm) is cut from each with a cylindrical cutter. The discs are put into a conditioning cabinet and after 20 min they are inflated on the Alveograph. An interval of about 1 min elapses between the inflation of successive discs, of which there are five for each dough.

Inspection of the quintuplet curves showed that the last tended to be the highest and shortest and that there were certain other consistent differences. The variations in the curves make it difficult to draw the mean curve, and it is desirable to reduce the variation. It appeared

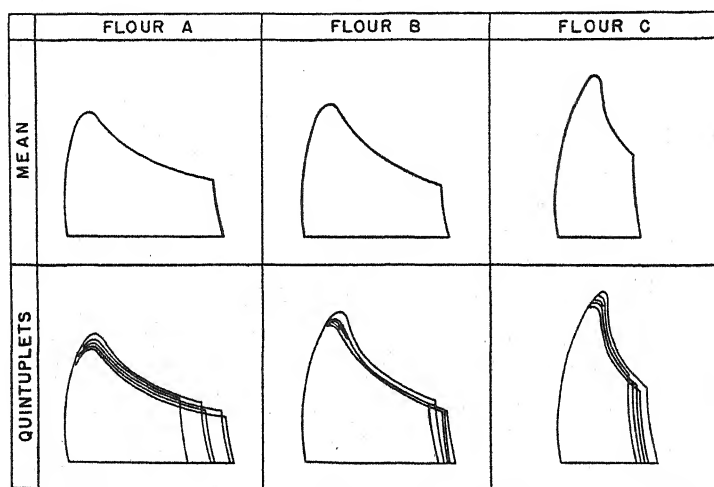


Fig. 4. Mean Alveograms (of quintuplet curves from eight doughs) for three flours, and representative groups of quintuplet curves for single doughs.

that it was caused by the longer mixing to which the last disc was exposed, or possibly to differences in the pressure at which the strip was extruded from first to last. But to test this hypothesis it was necessary to inflate the discs in reverse order so as to investigate the effect of the time factor. A study of reproducibility was also undertaken.

*Plan of Investigation.* The investigation was closely similar to that made with the Extensograph. The same three flours were used, and eight doughs were mixed, on the morning and afternoon of four different days. In place of the three Extensograph settings, two techniques, normal and reverse order of inflating the discs, were studied; and instead of duplicate Extensograms, quintuplicate Alveograms were made.

*Results and Discussion.* The mean curves for each flour, obtained by the normal order of testing, are shown in the upper half of Figure 4, and in the lower half are shown representative groups of curves for the quintuplet discs from one dough of each flour. The latter curves illustrate the type of variation obtained with the Alveograph.

Mean data for each disc, over all flours and replicates, are given for both orders in Table IV. These data show that the reverse order of testing did not change the rank order of the discs; the last disc tended to give the highest and shortest curve irrespective of whether it was inflated first (normal order) or last (reverse order). On the average, the length of the curve decreased from disc 2 to disc 5 in both normal and reverse procedures. There was also an increase in height from disc 3 to disc 5 in the reverse order; but in the normal order the third disc gave the lowest curve. Spreads between curves were considerably larger for the reverse than for the normal order, and this is certainly a point in favor of the normal order of testing. It is clear that, though the time between extrusion and inflation has some effect on the quintuplet curves from the same dough, the differences between the curves are caused principally by differences in the properties of the consecutive parts of the extruded dough. Accordingly, it seems reasonable to suggest that the whole technique of preparing the discs for inflation requires thorough revision.

TABLE IV  
MEAN DATA FOR NORMAL AND REVERSE ORDERS OF INFLATING DISCS

Disc No.	Length		Height	
	Normal	Reverse	Normal	Reverse
	<i>cm</i>	<i>cm</i>	<i>cm</i>	<i>cm</i>
1	9.3	9.2	10.0	9.8
2	9.3	9.2	9.8	9.8
3	8.8	8.7	9.7	9.8
4	8.4	8.1	9.9	10.2
5	8.2	8.0	10.5	11.0
Spread	1.1	1.2	0.8	1.2

As it is apparent that the normal order of testing is superior to the reverse order, the following discussion is confined to results obtained with the normal order.

The quintuplet curves can hardly be considered satisfactory replicates, and it seemed possible that an advantage might be gained by discarding three discs and considering the remaining two as duplicates. The data in Table IV suggested that the first two discs would prove most satisfactory, and inspection of the raw data confirmed this con-

clusion. A statistical comparison of the results of using the first two discs and all five discs showed that no real advantage could be gained. As would be expected, the standard deviation of measurements on a single curve from one dough decreased appreciably when only the first two discs were considered. But this was offset by a loss—with the majority of flours and measurements—in the precision of the mean data for one dough; the standard deviation for the mean of five discs for one dough tended to be lower than the standard deviation of the mean of the first two discs for one dough.

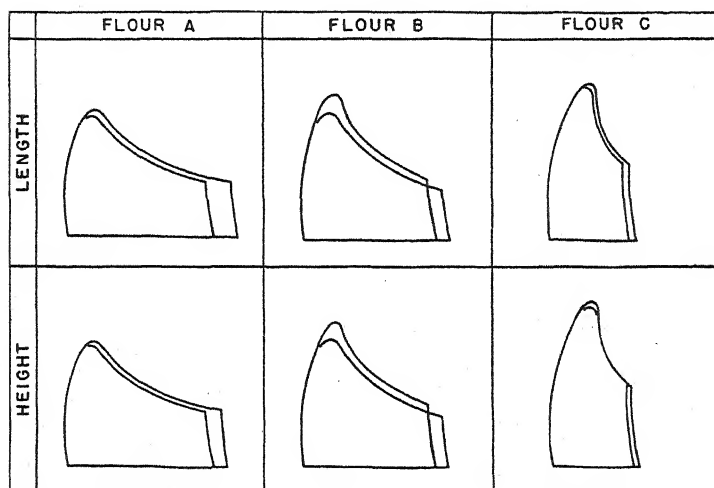


Fig. 5. Mean Alveograms (of quintuplet curves) showing the range in length and height obtained with eight replicate doughs.

The curves for the five discs made for one dough cannot be treated as replicates, and a discussion of the replicability must therefore be confined to that of the mean curve for separate doughs. Statistical analyses demonstrated that the differences between doughs examined on different days were no greater than those between doughs examined on the same day (with one negligible exception), and the eight doughs for each flour were therefore treated as representatives of one population.

Experimental errors of the mean curves for single doughs are illustrated in Figure 5, which contains a series of curves showing the maximum differences in height and length encountered with each flour. Corresponding data for the standard deviations of the mean curves are listed in Table V. The statistics show that a useful level of reproducibility is attained.



TABLE V

STANDARD DEVIATIONS FOR MEAN ALVEOGRAM MEASUREMENTS FOR ONE DOUGH

Measurement	Flour	Mean value	Standard deviation
		<i>cm</i>	<i>cm</i>
Length	A	11.0	0.4
	B	9.7	0.3
	C	5.7	0.2
Height	A	8.7	0.2
	B	9.8	0.3
	C	11.4	0.1

### Summary

The effects of three settings of the Extensograph on curve dimensions were investigated with duplicate curves for eight doughs from each of three flours. The setting has no effect on curve length, which measures the extensibility of the dough; but the height of the curve, which measures resistance to extension, increases as the machine is made more responsive. Most of the latter variation can be removed if resistance is reported in terms of the weight in grams required to produce the given curve height at the setting at which the machine is adjusted. The relation between curve height and resistance in grams can be made essentially linear at each setting. The standard deviations of measurements made on a single curve are of the following order: length,  $\pm 0.95$  cm; height,  $\pm 0.60$  cm; and resistance in grams,  $\pm 0.55$  g.

When curves are made on the Alveograph for the five discs cut consecutively from the extruded dough, the last disc gives, on the average, the highest and shortest curve. A study made with eight doughs from each of three flours shows that this happens whether the discs are inflated in normal or reverse order. The data show that though the time between extrusion and inflation has some effect on the quintuplet curves, the differences between these are caused principally by differences in the properties of the consecutive parts of the extruded dough strip. The quintuplet curves cannot be treated as replicates; replication can only be obtained by working with two separate doughs. It appears that the technique of preparing discs for the Alveograph requires thorough revision. The standard deviations for the mean of five curves made from a single dough are of the following order: length,  $\pm 0.3$  cm; and height,  $\pm 0.2$  cm.

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# SUSCEPTIBILITY OF BISCUITS TO INSECT DAMAGE <sup>1</sup>

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Hard biscuits of the type issued to the armed forces for emergency rations must frequently be stored for long periods, during which insect infestation may be an important cause of spoilage. The extent of such spoilage will be determined largely by the suitability of the biscuits for insect development and survival, and the development and survival of insects will be affected by the moisture content and the ingredients of the biscuits. If these factors are favorable to insects, then extensive damage may occur; and to the extent that they are unfavorable, the biscuits will be resistant to insect attack.

This study was designed to test the susceptibility of biscuits to insect damage when the moisture content and the principal biscuit ingredients were varied.

Before initiating the insect study it was necessary to determine the moisture equilibrium values for biscuits at different relative humidities. These determinations are reported in the first part of the paper; the second part reports experiments in which the confused flour beetle, *Tribolium confusum* Duv., was cultured on biscuits of varied composition and moisture content.

## Effect of Flour Grade and Fat Level on Moisture Equilibrium Values

The moisture content of dried foods is frequently a limiting factor for the development of insects feeding on them. Consequently, the effect of the principal constituents of the biscuits on the moisture equilibrium values at different relative humidities was given careful study.

Flour and fat are two important components in a typical ration biscuit formula and they are used in relatively high proportions; the former is the main basic ingredient and there is a tendency to use maximum amounts of the latter to increase the caloric value. Flour is hygroscopic and fat is not, and it is thus apparent that the proportions used will have an effect on the hydration capacity of the biscuits.

This section of the study deals with the moisture equilibrium values of biscuits processed from three grades of flour and three levels of fat when stored at three levels of relative humidity.

*Materials and Methods.* Two flours, one of 50% extraction and one

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of 85% extraction, were milled from a typical soft wheat of 10.2% protein content. A blend of equal parts of these flours provided a third flour of intermediate extraction. The protein and ash contents were 8.5% and 0.39% for the short-extraction flour, 9.0% and 0.66% for the blend flour, and 9.6% and 0.92% for the long-extraction flour.

The formula for a single batch of six biscuits was: flour, 100 g (13.5% moisture basis); cane sugar, 5 g; milk powder, 8 g; baking soda, 1.5 g; salt, 1.5 g; fat (hydrogenated peanut oil), variable; and distilled water, 38 ml. The fat levels were 2, 7, and 12 g. Biscuits were made by mixing all ingredients in a single-arm low-speed mixer (63 rpm) for 4 min, passing the dough through sheeting rolls three times, shaping with a cookie-cutter, and pricking the surface of the dough with a fork. The biscuits were baked on a wire-mesh mat for 12 min in an oven maintained at 230°C. This procedure gave an oval-shaped biscuit with dimensions of about 45 × 40 × 5 mm. Each batch of biscuits was cooled to room temperature and set aside in a closed container until the total number required was obtained.

Three desiccators, maintained at 25, 50, and 75% relative humidities by means of appropriate concentrations of sulfuric acid, were used as storage chambers. Each desiccator was equipped with three tiers of glass shelving, to support the moisture tins containing the biscuits, and a small two-bladed fan to circulate the air. The fan-shafts passed through a mercury-seal in the stopper of each desiccator and the three fans were propelled by a low-speed motor. The desiccators were maintained at a controlled temperature of 27°C. The initial moisture contents of the biscuits were determined by the A.O.A.C. vacuum oven method using random samples ground in a Wiley mill ( $\frac{1}{2}$  mm mesh sieve).

To determine the rates of moisture increase and the final moisture equilibrium values, triplicate samples of whole biscuits made from each of the nine formulae (three grades of flour and three levels of fat) were exposed to each of the three levels of humidity and weighed at intervals until constant weights were obtained. For the first few days, when the moisture increase was quite rapid, the weighing interval was 24 hr; later, the interval was extended to 48 hr or longer. The data were tabulated in terms of moisture content at each weighing, the values being computed from the original dry weight of each biscuit. As a check on the calculated equilibrium values, the final moisture contents of the biscuits were determined by the vacuum oven method.

To test the effect of aging, some of the biscuits were set aside in sealed tins at room temperature for a period of six months. The hydration capacity of these biscuits at the three levels of relative humidity was then determined in the same way as for fresh biscuits.

*Experimental Results.* The mean moisture data showing the moisture content of the fresh biscuits after exposure at the different humidities are summarized graphically in Figure 1 which shows the mean effect of fat level over all flours on the rate of moisture increase and on the equilibrium values, and in Figure 2 which shows similar comparisons for each flour over all fat levels.

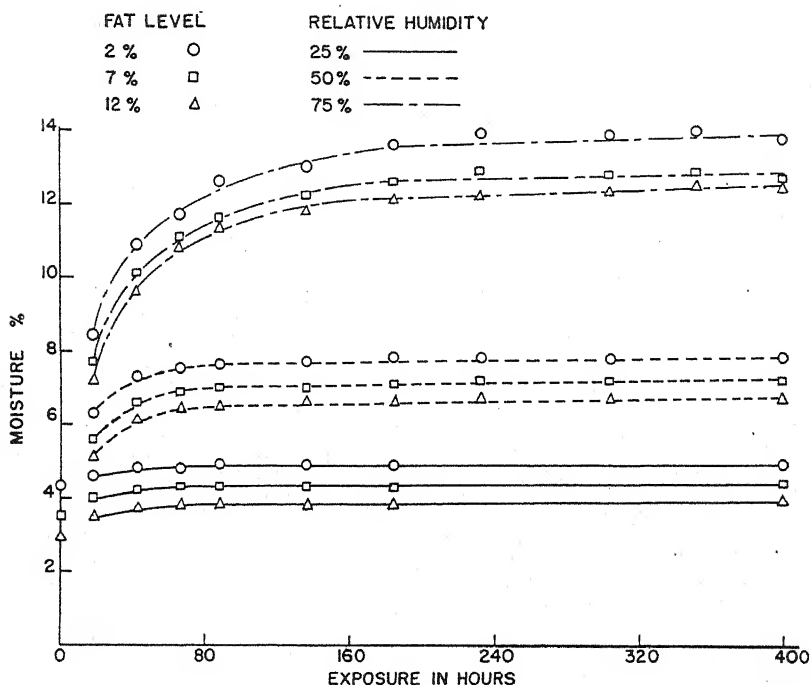


Fig. 1. The mean effect of fat content, over all flours, on the rate of moisture increase and the equilibrium moisture content of biscuits. The initial moisture contents of the biscuits are indicated by the points on the ordinate.

The moisture content of the biscuits increased quite rapidly during early exposure and then showed a drift to equilibrium. As the difference between the initial and final moisture content increased with each increase in relative humidity, the trend is obviously most apparent at the 75% humidity level. As would also be expected, equilibrium was attained more rapidly and at a lower level with each decrease in humidity.

The effect of increasing the fat content is clearly to lower both the initial moisture content and the equilibrium moisture content, at all levels of relative humidity. This effect is probably due to the displacement of the hygroscopic material, flour, by the nonhygroscopic material, fat. It is further apparent from Figures 1 and 2 that fat

content exerted a greater influence on moisture content than did flour grade. For example, the biscuits made from the long-extraction flour exposed to 75% humidity attained an equilibrium of 13.7% moisture for the 2% fat level, as compared with 12.0% moisture for the 12% fat level. At the 12% fat level, the corresponding values for the short-extraction and long-extraction flour biscuits were 12.8% and 12.0% respectively. Thus the spread in moisture content induced by the fat variable was more than twice that induced by the flour variable.

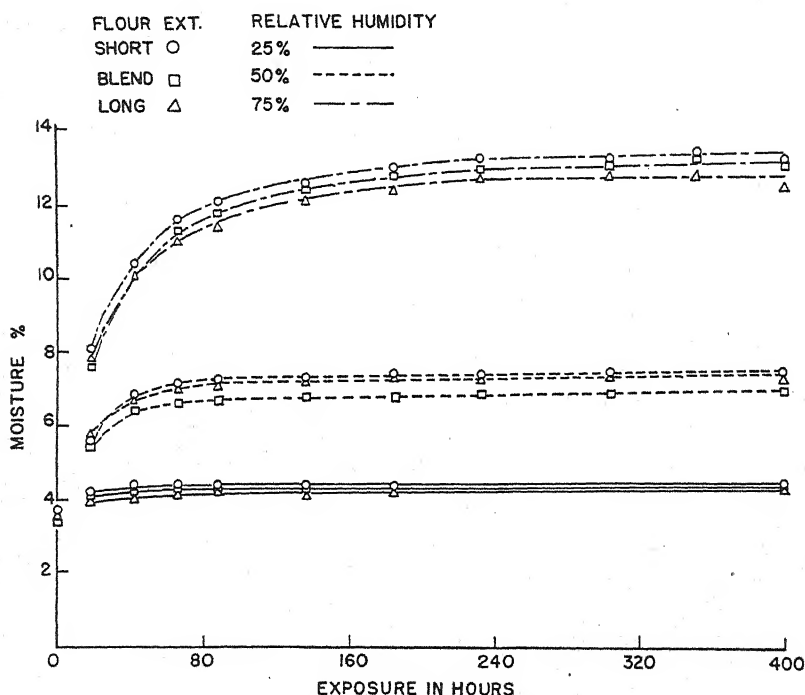


Fig. 2. The mean effect of flour extraction, over all fat levels, on the rate of moisture increase and the equilibrium moisture content of biscuits. The initial moisture contents of the biscuits are indicated by the points on the ordinate.

Figure 3 compares the hydration capacity of fresh biscuits at two levels of fat content with similar values for biscuits six months old. At relative humidities of 50% and 75% the hydration capacity of aged biscuits is reduced as compared to fresh biscuits, while at 25% relative humidity it is increased somewhat by aging. The effect of fat content remains the same for aged biscuits as for fresh biscuits. Typical values for fresh and aged biscuits (2% fat level) are: 4.9% and 5.6% at 25% relative humidity; 8.0% and 7.4% at 50% relative humidity; and 13.7% and 12.3% at 75% relative humidity.

### Effect of Moisture Content, Fat Level, and Flour Grade on Insects

A reliable measure of the susceptibility of a foodstuff to insect damage is the rate of development of insects feeding on it. Another measure is the percentage survival of the insects. Both measures relate to the fact that the damage caused by insects is a function of their numbers; and the number of insects also determines the possible extension of the range of infestation. Thus, insects gaining access to

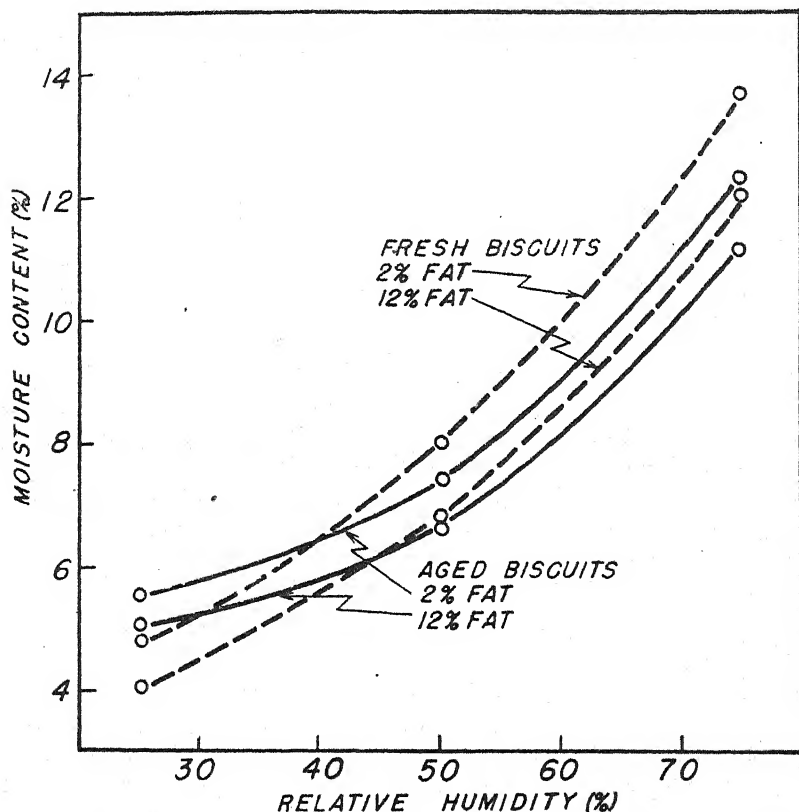


Fig. 3. The hydration capacity of fresh biscuits and biscuits aged for six months.

stored foodstuffs may achieve large numbers and establish a general infestation only when the foodstuff is favorable for their development and survival. This section of the paper deals with the ability of a common stored products insect to develop and survive on biscuits of the same type, and stored under the same conditions, as those described in the preceding section.

The details of the development of certain common insects on raw flour are known, but there is no similar information for insects on

processed flour in the form of biscuits, crackers, etc. The first object of these experiments, therefore, is to compare the development and survival of insects on flour and on biscuits. The second object is to assess the effect on insects of varying the moisture content, flour extraction, and fat level of the biscuits. High moisture content and long-extraction flours are known to favor insect development, but the effects of baking the flour and of fat content are largely unknown. The results indicate that the development and survival of insects on biscuits is greatly reduced as compared to their development and survival on the normal food, flour; and that at the low levels of development and survival obtained on biscuits, a high fat content exerts an unfavorable influence, while moisture content and flour extraction exert an influence in the same direction as with unbaked flour.

*Materials and Methods.* The confused flour beetle, *Tribolium confusum* Duv., is a cosmopolitan pest of cereal foods, and was chosen as the test insect in these experiments. Fifteen newly-hatched larvae were placed on each of 81 whole biscuits, comprising triplicate samples of each of the nine formulae (three grades of flour and three levels of fat) at three levels of humidity. At the same time, a sample of high-grade household bread flour was exposed to each humidity level and supplied with 10 newly-hatched larvae in two of the samples and 15 in the third. To test the effect of making the food more readily available, finely-ground biscuits of 2% and 12% fat content were also exposed to the three humidities, and each was supplied with 15 newly-hatched larvae. A constant temperature of 27°C was maintained.

The biscuits and controls were examined periodically. The development of larvae on flour and ground biscuit was followed by sifting off the flour and spreading out the ground biscuit to expose the larvae. On the whole biscuits, the larvae concealed themselves in the perforations or penetrated to the inside, so that very few were seen in the course of the experiment.

The experiment was discontinued after 113 days. Each biscuit was carefully broken up by hand, and the number, location, and size of the larvae noted. Three adult beetles of normal size were found inside the biscuits.

Since less than 1% of the original larvae achieved maturity on the biscuits, it was necessary to devise a measure of relative development other than the usual one of the time required to reach maturity. Since adults are actually shorter and weigh less than mature larvae, it is impossible to construct a continuous numerical scale for both larvae and adults in the order of their development.

In order to assess the central tendency in a group containing both adults and larvae at various stages of development, a scale of develop-

ment was devised based on larval size and the achievement of maturity. The surviving larvae were assigned to six size-classes on the basis of their length, according to the measurements of Brindley (1930) for six larval instars. The size-classes were arranged in order of increasing size and successive classes were progressively weighted by unity over the preceding class. This gave a scale of arbitrary values from 1 to 6 on the basis of larval size; adults were assigned a value of 7. The mean of the frequency distribution for larvae and adults according to their values on this scale of development is referred to as the "mean index of development" and provides a method for comparing relative development in groups containing both adults and larvae of various sizes.

The "mean index of development" cannot be interpreted in terms of larval instars or growth stadia. On unfavorable food, such as the biscuits, *Tribolium* larvae may pass through considerably more than the six instars described by Brindley (1930) so that there may have been several instars in any one of the size-classes used in calculating the mean index of development. But where the number of instars is variable because of unfavorable food (Good, 1933), larval size offers a better criterion for assessing the degree of development than the number of moltings. The "mean index of development" as used in this study refers only to insects that survived the 113 days of the experiment; those that died prior to this time were either shrunk so that measurement was impossible or had been eaten by the surviving insects.

*Experimental Results.* The effect of feeding on flour and on biscuits is sharply reflected in the development and survival of *Tribolium* larvae. Of 810 larvae placed on whole biscuits only three individuals or 0.4% achieved maturity during 113 days, while all larvae on flour achieved maturity in an average period of 48 days. On whole biscuits, 6.7% of the insects survived 113 days and achieved a mean development of 4.2, on the developmental scale of 1 to 7. On flour, 88.6% of the insects survived 113 days; all survivors were adults (developmental value of 7) and those that died were adults. The data are shown in Table I. The biscuits at 25% relative humidity were ex-

TABLE I  
DEVELOPMENT AND SURVIVAL OF FIRST-INSTAR LARVAE ON FLOUR,  
WHOLE AND GROUND BISCUITS AT 27°C

Larvae	Flour <sup>1</sup>	Biscuits <sup>2</sup>	
		Whole	Ground
Original number	35	810	60
Percent surviving	88.6	6.7	5.0
Percent achieving maturity	100	0.4	0.0
Mean index of development	7	4.2	3.7

<sup>1</sup> Data after 48 days.

<sup>2</sup> Data after 113 days.



cluded from these comparisons because their moisture content was lower than that of the flour controls.

On ground biscuits, the values for development and survival are about the same as for larvae on whole biscuits. Accordingly, the observed retardation of development and the high mortality of *Tribolium* larvae cannot be attributed to the inability of the larvae to obtain food from the whole biscuits.

Considering the biscuits alone, the effects of moisture content, flour extraction, and fat content may be discerned by comparisons of the development and survival of the 57 insects that remained after 113 days. The data were grouped successively to show the effect of moisture content over all biscuit formulae, flour extraction over all humidities and all fat levels, and fat level over all humidities and all flours. The data are shown in Figure 4.

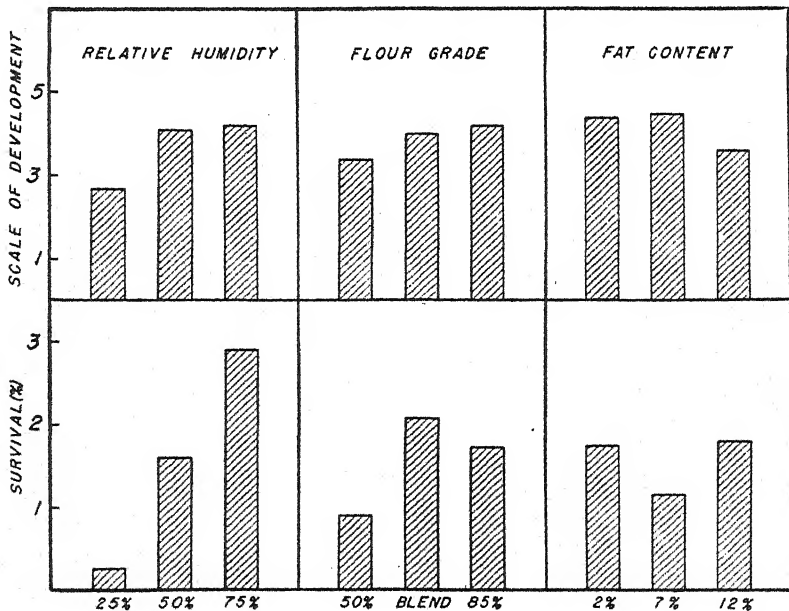


Fig. 4. The mean effect of relative humidity, flour grade, and fat content on the development and survival of larvae reared on biscuits for 113 days at 27°C.

The effect of humidity, or the moisture content of the biscuits (Fig. 4), is to reduce the survival of insects progressively as the relative humidity is lowered from 75% to 25%. The development of larvae is retarded in the same way, very slightly between 75% R.H. and 50% R.H., but abruptly at 25% R.H. On biscuits at 25% R.H. only three larvae survived to achieve a very small growth, at 50% R.H. there was

one adult, and at 75% there were two. The moisture contents of biscuits at 75%, 50%, and 25% relative humidity ranged from 11.8% to 14.1%, 6.5% to 8.1%, and 3.6% to 5.1%, respectively.

The flour grade used in making biscuits influences insect development, long-extraction flour favoring development and refined flour retarding it (Fig. 4). Two larvae reached maturity on biscuits made with a flour of 85% extraction, and one reached maturity on biscuits of 50% extraction flour. Although no larvae reached maturity on biscuits made with a blend of the two flours, there were more in an advanced stage of growth on biscuits made with the blend than on biscuits of 50% extraction flour. Biscuits made with the 50% extraction flour were more unfavorable for insect survival than the other two. Survival appeared to be slightly favored on biscuits made with a blend of the two flours.

The fat content of the biscuits appears to have little effect on the survival of *Tribolium* larvae (Fig. 4). Development however is adversely affected by a fat content of 12%. Biscuits of 7% fat content appear to favor development slightly over biscuits with a fat content of 2%. Two larvae reached maturity on biscuits of 7% fat content and one reached maturity on biscuits of 2% fat; on biscuits of 12% fat there were no adults, and the mean growth of larvae was less than that achieved on biscuits of lower fat content.

The fat content of the biscuits seems to play an important mechanical role in resisting insect attack. Biscuits of 12% fat content are of closer texture and lack the fragile "blisters" that are characteristic of biscuits with a lower fat content. Insect larvae are able to penetrate these blisters and gain access to the inside of the biscuits where they seem to develop more rapidly. The three adult beetles were found *inside* the biscuits, and so was the largest larva. The influence of fat content on larval penetration is shown in Table II.

TABLE II  
EFFECT OF FAT CONTENT OF BISCUITS ON PENETRATION OF LARVAE

	2% fat	7% fat	12% fat
Surviving larvae, number	21	15	22
Larvae inside, number	7	6	2
Larvae inside, %	33	40	9

The damage caused by the insects was very slight because so few of them survived to reach an advanced stage of development. The larvae penetrated "blisters," and widened crack-lines on the biscuit surface, often excavating a small cavity in these cracks. Damage of this sort was much more apparent on the biscuits of low fat content

than on those with a high fat content. The cracks and excavations on the low-fat biscuits were increasingly widened and deepened as the experiment continued; but the damage to the high-fat biscuits was scarcely noticeable at the end of the experiment.

### Discussion

Biscuits of the type described are unfavorable for the development and survival of *Tribolium* larvae. The results show that, no matter how the moisture content or the principal components of the biscuits are varied, there is a very high mortality of larvae reared on the biscuits, and the development of the few survivors is greatly retarded by comparison with the rate of development on flour. The differences in mortality and development, between larvae reared on flour and those reared on biscuits, are much greater than the differences induced by varying the moisture content and constituents of the biscuits themselves. It is, therefore, reasonable to suppose that merely baking the flour, in the process of biscuit-making, renders it an unsuitable food for the insects. The baking of flour is known to result in loss of thiamine; and thiamine is indicated as a necessary factor for the normal development of *Tribolium* (Street and Palmer, 1935). Moreover, severe losses of thiamine are reported for biscuits baked on the alkaline side (Barackman, 1942), and the basic formula for biscuits used in this study called for 1.5 g of baking soda and no acid salt. Accordingly, it appears that the observed retardation of development and high mortality of *Tribolium* on these biscuits may be related to losses of thiamine during the baking process. This phase of the problem is now in the course of investigation.

The moisture content of the food is an important factor for insect pests of stored products. Both the survival and development of *Tribolium* larvae are reduced as the moisture content of the biscuits is lowered. However, the moisture content of the food is of much less importance than the food itself. For example, all larvae reared on flour of 8.8% moisture content (25% R.H.) completed development, while on biscuits of comparable moisture content (7%–8%) only one of 405 larvae reached maturity. Obviously, the most important factor for *Tribolium* feeding on the biscuits is the nutritional deficiency induced by baking. Superimposed on this major factor is the usual retarding effect when the moisture content of the food is reduced.

The reduction of moisture content obtained by increasing the percentage of fat in the biscuits has no very great effect on *Tribolium*, since the insect has a wide tolerance in moisture requirement, and the reduction of moisture content is small, even for relatively large additions of fat. All larvae achieved maturity on flour within the range

of 14.2% to 8.8% moisture content; and the greatest reduction in the moisture content of the biscuits, obtained by a 10% increase in fat level, was 1.7%. This reduction in moisture content would be chiefly effective against insects on biscuits stored in dry atmospheres.

The observation that at relatively high humidities the hydration capacity of the biscuits decreases with aging indicates that biscuits of this type will become somewhat more resistant to insect attack during storage. The reduction of hydration capacity with age is to be expected since loss of imbibition is a characteristic of staling (Steller and Bailey, 1938); but this does not explain the increase in the hydration capacity of aged biscuits at 25% R.H.

The favorable effect of long-extraction flours over refined flours has been demonstrated for a number of different cereal pests, including *Tribolium* (Good, 1933; Park, 1934; Thomas and Shepard, 1940). The basis for this effect is the absence, in refined flours, of sufficient quantities of minerals and B-complex vitamins. Many of the B vitamins are essential for the normal development of insects that infest stored products (Fraenkel and Blewett, 1943). When flour is baked to produce biscuits, the favorable effect of long-extraction flour over refined flour is still apparent, although at a much lower level than for unprocessed flour. As already suggested, thiamine is the substance that is probably affected by baking. Apparently, the destruction of thiamine during baking is parallel for both long-extraction and short-extraction flours. Biscuits made with a long-extraction flour are therefore more favorable for insects than those made with a short-extraction flour because the thiamine content of the former is higher initially.

The fat content of the biscuits appears to retard development but has no effect on survival. Sweetman and Palmer (1928) found that on an otherwise adequate diet the addition of 10% butterfat or crisco actually accelerated the growth of *Tribolium*. Thomas and Shepard (1940) found that ground walnuts of high oil content were fatal to larvae of the saw-toothed grain beetle, but chopped walnuts had no detrimental effect. In the present study there are no indications that high-fat biscuits, whether whole or ground, are more unfavorable to survival than biscuits of lower fat content.

### Summary

Hard biscuits of the type issued to the armed forces, made from three grades of flour and three levels of fat, were stored at three levels of relative humidity to determine the moisture equilibrium values. Increasing the fat content of the biscuits lowered both the initial and the equilibrium moisture content at all levels of relative humidity. Flour grade exerted less influence on moisture content than did fat

content. Aging the biscuits decreased their hydration capacity at high relative humidity and increased it at low relative humidity.

Larvae of the confused flour beetle, *Tribolium confusum* Duv., were reared on the biscuits at three levels of relative humidity. Both the rate of development and the percentage survival of *Tribolium* larvae reared on biscuits were adversely affected as compared to flour controls. Development was greatly retarded and mortality was high for larvae reared on both whole and ground biscuits. It is suggested that destruction of thiamine during the baking process is responsible for the high resistance of the biscuits to insect attack. At the low level of survival and development attained by *Tribolium* larvae on biscuits, high moisture content and biscuits made with a long-extraction flour favored the insects, while biscuits of high fat content were somewhat unfavorable. A high fat content rendered the biscuits less susceptible to penetration by *Tribolium* larvae.

#### Acknowledgments

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# THE RELATIVE GERMICIDAL EFFECTIVENESS OF CERTAIN CHLORINE COMPOUNDS ON THE THERMODURIC BACTERIAL SPORES OF WHEAT

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The causative organism of ropiness in baked wheaten products has been variously identified as *B. mesentericus pani viscosus I and II* (Vogel, 1897), *B. mesentericus vulgatus* (Russell, 1898), *B. mesentericus fuscus* (Watkins, 1906), *B. panis* (Fuhrmann, 1905), and *B. mesentericus* (Kent-Jones and Amos, 1930). In any event, the one or more organisms belong to the *B. subtilis* group comprising many widely distributed soil-inhabiting sporulating thermophilic bacilli. It is fairly well agreed they contaminate all wheat, and in the normal milling process they are partly carried into the flour, remaining dormant there, surviving the baking process and exhibiting their presence in the loaf the first or second day following the bake when the interior of the loaf partially liquefies and a foul odor develops.

Since the growth of the bacilli is inhibited by slightly increasing the acidity above that normally encountered in bread, vinegar has long been used as an ingredient of bread during the summer when infection is most likely to appear. Recently, sodium and calcium propionates<sup>1</sup> and sodium diacetate<sup>2</sup> have been utilized for their claimed superior bacteriostatic action.

More basic is the treatment applied within the last few years at the mills. Solutions of chlorine-containing compounds are now used in treating the wheat prior to milling, resulting in a great reduction or actual elimination of rope and mold spores on the wheat. In treating the wheat, advantage is taken of the universal practice of adding 3-4% water to improve the milling qualities. Into the stream of water being blended into the wheat sufficient germicidal solution is fed to provide an available chlorine content of 50-100 ppm, based on the water added. The wheat after being tempered is held in bins for 18-24 hr so that adequate time is allowed for the completion of bactericidal action if sufficient active agent remains present. Because the chloramines are generally more resistant to decomposition in the presence of organic matter, it seemed desirable to determine their relative value in such an application as this.

The object of this work was to ascertain on a laboratory basis the

<sup>1</sup> (Mycobans) E. I. duPont de Nemours and Company, Inc., Wilmington, Del.

<sup>2</sup> Niacet Chemicals Corporation, Niagara Falls, N. Y.

relative germicidal efficiency of Chloramines B and T and a commercial hypochlorite product.

### Materials and Methods

The composition of the hypochlorite on analysis of an open market sample was calculated as equivalent to  $\text{Ca}(\text{OCl})_2$ , 17.3%;  $\text{Ca}(\text{OH})_2$ , 9.0%;  $\text{CaCl}_2$ , 6.3%;  $\text{Na}_2\text{CO}_3$ , 66.5%; undetermined, 0.9%. On dissolving in water, sodium hypochlorite is liberated in solution by the reaction



The available chlorine content of the hypochlorite tested was 17.2%. The Chloramines B and T used were of U.S.P. or equivalent grade.

As all wheats, regardless of source, carry a population of the spores under consideration, wheat samples chosen at random may be treated with dilute solutions of germicide and the reduction in population estimated by means of a method such as that of Kent-Jones and Amos (1930). This method is much used by cereal workers, but is rather inaccurate both because of its nature and because of the small number of spores naturally present in normal wheat. First quality cleaned wheat will bear only from 1 to 25 and rarely as high as 50 spores per g, significant commercially, but very low figures for bacteriological test purposes. The test depends on the determination of the smallest amount of wheat which gives in the average case a positive test for presence of spores. When germicidal action is nearly complete, objectionably large portions of ground wheat must be added to the test medium in order to obtain a positive result. Owing to the chance distribution of spores and the other limitations of the method, it is obvious that great variability in results may be expected, and that has been our experience.

By sacrificing some of the *in vivo* aspects of the Kent-Jones and Amos test and substituting more conventional bacteriological methods, a greater degree of precision may be obtained. Pure cultures of the organism were used in the following work and details of their preparation and the test methods used follow.

Several samples of wheats were cultured in nutrient broth, boiled 30 min to destroy all but the thermoduric spores, and incubated. From tubes showing typical pellicles, transfers were made to nutrient agar plates. The best colonies were allowed to grow until the drying of the agar and the overcrowding of the colony were deemed to have encouraged the formation of large numbers of spore forms. Surface scrapings of each selected colony were separately mixed by mortar and pestle with a diluent of sterile silica flour, 250–325 mesh, and heated at 100°C for 20 min to destroy any vegetative forms and any foreign

bacteria possibly included during the operation. The actual concentrations of spores in these silica dilutions were not obtained, but were very high; 5 mg of the material added to 25 ml of liquid insured positive inoculation of culture tubes when only 2 drops of this liquid dilution were used.

Two pure varieties of thermoduric, spore-forming organisms thus were obtained and used as test organisms for the following work. Their cultures on agar plates, in nutrient broth, on potato slants, and microscopic examination indicated that they were strains of *B. subtilis* and *B. mesentericus*. It was demonstrated that the organisms isolated were capable of causing ropiness in baked wheaten products.

Tubes were set up at room temperatures approximating 30°C containing 25 ml of the desired concentrations of germicides, and to each was added approximately 5 mg of the silica flour mixture of the spores. At definite intervals, 2 drops of the solution were added to tubes of nutrient broth, which were immersed in boiling water for 25 min, cooled, and incubated at 30°C for 48 hr. Tubes then showing formation of a pellicle were reported as positive.

### Results

In Tables I and II are given the data on the action of various germicides, without the addition of sterile wheat flour in Table I, and

TABLE I

EVALUATION OF BACTERICIDES ON THE SPORES OF *B. SUBTILIS* AND *B. MESENTERICUS*

Concentration of available chlorine in ppm	Germicidal agent					
	Chloramine B		Chloramine T		Sodium hypochlorite	
	200 sec	500 sec	200 sec	500 sec	200 sec	500 sec
<b>B. SUBTILIS</b>						
0	+	+	+	+	+	+
25	+	+	+	+	+	+
50	+	+	+	+	+	—
75	+	—	+	—	+	—
100	—	—	—	—	—	—
150	—	—	—	—	—	—
<b>B. MESENTERICUS</b>						
0	+	+	+	+	+	+
25	+	+	+	+	+	+
50	+	—	+	+	+	+
75	—	—	+	—	+	+
100	—	—	—	—	—	—
150	—	—	—	—	—	—



TABLE II  
EVALUATION OF BACTERICIDES ON THE SPORES OF *B. SUBTILIS* AND  
*B. MENTERICUS* IN THE PRESENCE OF 50 PPM WHEAT FLOUR

Concentration of available chlorine in ppm	Germicidal agent					
	Chloramine B		Chloramine T		Sodium hypochlorite	
	200 sec	500 sec	200 sec	500 sec	200 sec	500 sec
<i>B. SUBTILIS</i>						
0	+	+	+	+	+	+
25	+	+	+	+	+	+
50	+	+	+	+	+	+
75	+	-	+	+	-	+
100	+	-	-	-	-	-
150	-	-	-	-	-	-
<i>B. MENTERICUS</i>						
0	+	+	+	+	+	+
25	+	+	+	+	+	+
50	+	+	+	+	+	-
75	+	-	+	+	+	-
100	-	-	-	-	+	-
150	-	-	-	-	-	-

with its addition in Table II, to provide some added organic matter in the system similar to that which in commercial practice is always present.

No differences in resistance to germicidal action beyond those of experimental error of the method seem to exist between the test organisms. The addition of small amounts of wheat flour has shown only the most minor effect though it is possible that quantities of greater magnitude might exert some unfavorable effect.

Finally, the three chlorine germicides, Chloramine B, Chloramine T, and sodium hypochlorite, have averaged almost precisely equal in efficiency when used at the same available chlorine level.

Seventy-five ppm available chlorine is about the critical concentration for 500 sec exposure and 100 ppm must be used for assurance of complete killing. One hundred ppm available chlorine is about the critical concentration for 200 sec exposure and 150 or possibly 125 ppm is required for assurance of complete killing within this shorter period.

### Summary

Three germicides have been evaluated for their action on the spores of thermophilic bacteria of wheat, tests being made on the spores in pure cultures.

Sodium hypochlorite, Chloramine B, and Chloramine T compared at equal levels of available chlorine are approximately equally effective towards the organisms tested.

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### SOME FACTORS AFFECTING THE DETERMINATION OF RIBOFLAVIN BY THE FLUOROMETRIC METHOD<sup>1</sup>

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Fluorometric methods for the assay of riboflavin have come into wide use for production control purposes as they are more rapid and convenient than the microbiological procedure. The collaborative studies of the Subcommittee on Riboflavin Assay of the American Association of Cereal Chemists (Andrews, 1943, 1943a) have demonstrated that while these two types of assays yield comparable average values, wide variations occur in the results of individual collaborators for each of the methods. Andrews (1943b) has shown that the assay values by both procedures decrease as the quantity of sample taken for analysis is increased. The second collaborative study (Andrews, 1943a) indicated that variations in the efficiency of different lots of "Florisil" to adsorb riboflavin might be a possible source of error in the fluorometric procedure investigated by the subcommittee and it was recommended that the efficiency of Florisil should be checked by adding riboflavin to cereal extracts under study and determining the amount which can be "recovered" by assay. Oxidation with permanganate to destroy interfering pigments was found to be essential in

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the assay of bread but from preliminary experiments it appeared to be unnecessary for enriched flour.

These collaborative studies have shown that the assay of riboflavin is not a simple problem and that there is a need for a detailed investigation of the various steps in the fluorometric procedure. During the course of experiments conducted with the object of developing a rapid fluorometric technique for routine mill control work, some studies were made of the effect of size of sample, of the efficiency of adsorption on Florisil, and of the choice of light filters on assay values. These are presented in this paper.

### Experimental

*The Reference Method.* The assay procedure employed as a standard of reference in these experiments was based principally on the work of the Subcommittee on Riboflavin Assay (Andrews, 1943 and 1943a) with some changes suggested by our own experience. The essential features of the reference method are as follows:

Suspend 1 g of sample in 50 ml of 0.1N  $\text{H}_2\text{SO}_4$  and digest for 1 hr at the temperature of boiling water. Cool, add 12 ml of 6.5%  $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$  and 2 ml of glacial  $\text{CH}_3\text{COOH}$ . Make up to 100 ml with water, add 1 g of Celite and filter. To a 50-ml aliquot of the clear extract add 1 ml of 4%  $\text{KMnO}_4$  solution, followed, 1 min later, by 1 ml of 3%  $\text{H}_2\text{O}_2$  solution. The adsorption, elution, and measurement of the riboflavin are carried out as described by Andrews (1943a).

*Effect of Sample Size and Florisil Efficiency.* Extracts were prepared from 1, 3, 6, and 10 g of second clear flour (Sample No. 1, ash content 1.2%) as outlined in the reference method. Fifty ml of each filtered extract was passed in succession through a series of four adsorption tubes containing 1.1 g of Florisil (Lot No. 1) and the riboflavin adsorbed by each tube was determined. In a parallel experiment, different volumes of one extract (10 g of flour to 100 ml) were each passed through a series of four adsorption tubes and the riboflavin which was taken up in each tube determined. These experiments were repeated later with a new lot of Florisil (No. 2) and another sample of clear flour (No. 2).

The results for equal volumes of clarified extracts representing various weights of flour are recorded in Table I. As the sample size is increased, a smaller fraction of the total riboflavin of the extract is adsorbed in the first tube. Florisil No. 1 required three tubes to adsorb all the riboflavin when a 1-g sample was used, and a single tube adsorbed less than 50% of the riboflavin of the extracts prepared from 6 and 10 g of clear flour. Florisil No. 2 was much more efficient. Despite the fact that adsorption was apparently complete with this Florisil for all the extracts after they had been passed through the third tube, the total recovery decreased with increasing sample size

TABLE I

EFFECT OF SAMPLE WEIGHT ON ADSORPTION OF RIBOFLAVIN FROM CLARIFIED FLOUR EXTRACTS BY FLORISIL

Sample weight	Flour <sup>1</sup> equivalent in aliquot	Riboflavin adsorbed				
		Tube 1	Tube 2	Tube 3	Tube 4	Total
g	g	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
FLORISIL NO. 1						
1	0.5	0.95	0.11	0.05	0.00	1.11
3	1.5	0.57	0.24	0.12	0.03	0.96
6	3.0	0.45	0.26	0.16	0.07	0.94
10	5.0	0.44	0.24	0.14	0.09	0.91
FLORISIL NO. 2						
1	0.5	1.15	0.00	0.00	0.00	1.15
3	1.5	0.97	0.08	0.00	0.01	1.06
6	3.0	0.84	0.11	0.03	0.01	0.99
10	5.0	0.76	0.16	0.03	0.01	0.96

<sup>1</sup> The flours employed in preparing the extracts for use with Florisil No. 1 and No. 2 were different samples of clear flour.

so that some factor in addition to Florisil efficiency is apparently partly responsible for the sample size effect. Actually only about one half the lowering of the assay values, obtained by the use of single tubes, which result from taking larger sample weights can be accounted for by incomplete adsorption of the extracted riboflavin by Florisil No. 2. For example, the assay value for the 3-g sample is  $0.18 \mu\text{g/g}$  lower than that for the 1-g sample, and after complete adsorption a difference of  $0.09 \mu\text{g/g}$  still remains; similarly, the assay values for the 5- and 10-g samples, based on the results of single adsorptions, are  $0.31$  and  $0.39 \mu\text{g/g}$  lower than for the 1-g sample, while the results after complete adsorption are  $0.15$  and  $0.20 \mu\text{g/g}$  lower.

The results obtained when different volumes of the same clarified extract were each passed through a series of four Florisil tubes are recorded in Table II. Here the differences are due entirely to inefficient adsorption; as the aliquot was increased, more and more riboflavin escaped adsorption by the first tube. Dilution of the aliquots before adsorption had no effect on the results. If, however, 10 g of flour was digested with 50 ml of sulfuric acid and appropriate amounts of sodium phosphate, acetic acid, sulfuric acid, and water added to bring the volume to 1 liter instead of 100 ml and an aliquot oxidized, the same riboflavin values were obtained as when 1 g of sample was digested and diluted to 100 ml.

TABLE II  
EFFECT OF ALIQUOT VOLUME ON ADSORPTION OF RIBOFLAVIN FROM CLARIFIED  
FLOUR EXTRACTS<sup>1</sup> BY FLORISIL

Aliquot	Riboflavin adsorbed				
	Tube 1	Tube 2	Tube 3	Tube 4	Total
ml	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
FLORISIL NO. 1					
25	0.67	0.28	0.07	0.04	1.06
50	0.49	0.32	0.11	0.07	0.99
75	0.34	0.31	0.15	0.14	0.94
100	0.29	0.28	0.14	0.16	0.87
FLORISIL NO. 2					
5	0.92	0.03	0.00	0.00	0.95
15	0.92	0.05	0.01	0.00	0.98
30	0.83	0.13	0.02	0.00	0.98
40	0.76	0.13	0.05	0.01	0.95
50	0.76	0.16	0.03	0.01	0.96

<sup>1</sup> The flours employed in preparing the extracts for use with Florisil No. 1 and No. 2 were different samples of clear flour.

The results given in Tables I and II show that increasing the size of sample has two effects: first it decreases the efficiency of extraction and secondly it decreases the adsorption efficiency of Florisil. With Florisil No. 2, these effects were of approximately equal magnitude. A number of facts lead to the suggestion that soluble substances decrease the adsorption efficiency of Florisil in proportion to the quantities which are present in the clarified extracts. Even Florisil No. 1, which was of low efficiency, adsorbed riboflavin quantitatively from aqueous or buffered acid solutions as a sharp band near the top of the column. The lowered efficiency with flour extracts as the sample size is increased must therefore be ascribed to the presence of increasing quantities of impurities in the extract. That the amount rather than the concentration of these substances is the controlling factor is supported by the fact that dilution of extracts after clarification had no effect on the efficiency of adsorption.

To determine whether pure riboflavin added to a flour extract was adsorbed more readily than that naturally present, an extract of second clear flour (No. 2) was prepared using a sample extractant ratio of 7 g to 100 ml and divided into two equal portions after filtration. To one, pure riboflavin was added in the proportion of 1  $\mu\text{g}/10$  ml. The enriched and unenriched extracts were each passed through a Florisil tube (Florisil No. 2) and were collected in successive 10-ml

TABLE III

THE FLUORESCENCE OF SUCCESSIVE PORTIONS OF AN EXTRACT OF SECOND CLEAR FLOUR, WITH AND WITHOUT ADDED RIBOFLAVIN, AFTER PASSING THROUGH FLORISIL

Portion	Total of portions	Fluorescence <sup>1</sup>		Riboflavin adsorbed <sup>2</sup>	
		Without added riboflavin	With added riboflavin	Native riboflavin	Added riboflavin
	ml			%	%
1	10	0.0	0.5	100	100
2	20	1.0	1.0	97.5	100
3	30	1.5	1.0	95.8	100
4	40	1.5	1.0	95.0	100
5	50	1.5	1.5	94.5	100
6	60	3.0	3.0	93.0	100
7	70	4.5	5.0	90.7	100
8	80	6.5	6.5	87.8	100
9	90	8.5	10.5	84.4	99.2
10	100	10.0	14.0	81.0	97.9
11	110	14.0	21.5	76.4	95.8
Original Extract		20.0	49.0		

<sup>1</sup> Units of deflection of Coleman photofluorometer less blank.

<sup>2</sup> As percentages of the total quantities passed through the columns, calculated as follows:

$$\% \text{ Native riboflavin adsorbed from 110 ml} = \frac{100 \times [(11 \times 20) - a]}{220} = 76.4$$

$$\% \text{ Added riboflavin adsorbed from 110 ml} = \frac{100 \times [(11 \times (49 - 20))] - (b - a)}{11 \times (49 - 20)} = 95.8$$

Where a equals the sum of the values in column 3, and b the sum of the values in column 4.

portions, the fluorescence of which was measured with the results shown in Table III.

Added riboflavin was completely adsorbed until 80 ml of the extract (containing 8  $\mu$ g of added riboflavin) had passed through the column. Of the 5.5  $\mu$ g of native riboflavin in this volume of extract, only 87.8% was adsorbed. It is thus evident that pure riboflavin added to a flour extract may be quantitatively adsorbed, while the riboflavin native to the extract is only partially adsorbed. When more than 80 ml of the solutions were used, it was found that the enriched extract contained more riboflavin after adsorption than the unenriched extract from the other tube, indicating that some of the added riboflavin was leaking through the Florisil. Other experiments showed that the recovery of riboflavin added to filtered second clear extracts decreased as the concentration of the extract increased.

When riboflavin was added to the mixture of flour and extractant either before digestion, or after digestion and cooling, the recoveries were lower than when the addition was made to the clarified extracts. The low recoveries which may be obtained when varying amounts of riboflavin are added to 10 g of second clear flour, either before extraction, or to the flour and extract before dilution, will be seen from the results in Table IV. While the extract was concentrated, only 10 ml

was passed through a Florisil tube in each case. Under these conditions all the added riboflavin, and all except about 0.04  $\mu\text{g}$  of the native riboflavin actually in solution, is adsorbed on the Florisil. Sample No. 2 was used in this experiment and, as shown in Table I, this flour gave a result of 1.15  $\mu\text{g}$  of riboflavin per gram when 1 g was taken for assay.

TABLE IV  
RECOVERIES OF RIBOFLAVIN ADDED TO 10 G OF FLOUR BEFORE AND AFTER DIGESTION

Added	Found	Recovery	Riboflavin total Native <sup>1</sup> + added	Total in extract	Total adsorbed on flour
$\mu\text{g/g}$	$\mu\text{g/g}$	%	$\mu\text{g}$	$\mu\text{g}$	$\mu\text{g}$
RIBOFLAVIN ADDED BEFORE EXTRACTION					
0.0	0.96	—	11.5	9.6	1.9
1.0	1.80	84.0	21.5	18.0	3.5
2.0	2.69	86.5	31.5	26.9	4.6
3.0	3.47	83.7	41.5	34.7	6.8
RIBOFLAVIN ADDED AFTER EXTRACTION					
0.0	0.93	—	11.5	9.3	2.2
1.0	1.87	94.0	21.5	18.7	2.8
2.0	2.75	91.0	31.5	27.5	4.0
3.0	3.60	89.0	41.5	36.0	5.5

<sup>1</sup> The flour was found to contain 1.15  $\mu\text{g}$  of riboflavin when a 1-g sample was taken for assay.

The low recoveries of added riboflavin were due to the operation of the "extraction factor" which appears from Table IV to be nothing more than the adsorption of native or added riboflavin by the flour residues which are left on the filter paper. The riboflavin thus adsorbed can be released by dilution.

*Filters.* After testing various Corning glass filters, Conner and Straub (1941) recommended the use of filter 511 for incident light and filter 351 for fluorescent light. They found that an aqueous solution of riboflavin gave the highest fluorescence readings and the lowest blank with this combination of filters. In our assays it proved unsatisfactory. Pyridine eluates are extremely sensitive to ultraviolet light, and their fluorescence dropped very rapidly when filter 511 was used alone for the incident light. On opening the shutter, the galvanometer needle swung over to a maximum and then immediately began to fall off. It seemed certain that all our readings were slightly low and, what was more important, that the error depended upon the variable time required to get a reading after opening the shutter.

A more serious difficulty was that of determining the blank for unenriched patent flours. Each addition of sodium hydrosulfite, after the first one, caused a small decrease in the fluorescence so that it was impossible to select any particular reading as the blank. Eventually both difficulties were overcome by using Corning filter 038 in combination with 511 for the incident light as recommended by Andrews (1943). By means of a Coleman spectrophotometer, set for a wavelength of 445  $m\mu$ , it was found that filter 038 transmitted only 50% of the incident light. When screened from the light source by filter 038, the fluorescence of pyridine eluates decreased far less rapidly, while the uncertainty as to the blank readings for flour eluates also disappeared.

Since others have experienced trouble in getting satisfactory blank values (Andrews, 1943 and 1943a), the results of an experiment to show the effect of using filter 038 are reported. Additions of a 5% solution of sodium hydrosulfite in ice-cold water were made to 10 ml of a pyridine eluate from flour, and the instrument reading, without filter 038, was taken after each addition. The filter was then inserted and the experiment repeated with another portion of the same eluate. An eluate from a sample of enriched bread was treated in the same manner. The results are shown in Table V.

TABLE V  
EFFECT OF ADDITIONS OF SODIUM HYDROSULFITE ON THE FLUORESCENCE READINGS OF PYRIDINE ELUATES WITHOUT AND WITH FILTER 038

Hydrosulfite added	Fluorescence readings <sup>1</sup>			
	Flour		Bread	
	Filter 038		Filter 038	
	Out	In	Out	In
mg				
10	16.2	6.1	10.1	4.5
20	12.4	5.5	8.2	4.1
30	10.5	5.3	7.4	3.7
40	9.1	4.8	7.3	3.7
50	8.7	4.5	6.9	3.5
70	6.8	4.0	5.7	3.4
125	5.0	3.5	5.0	3.1
estimated blank	?	4-5	6-7?	3.5-4.0

<sup>1</sup> Units of deflection of Coleman photofluorometer corrected for dilution; ordinarily the instrument is read to the nearest 0.5 units.

The difficulty of selecting the blank reading for the flour eluate without filter 038 will be obvious. When the filter was used, however, the range of uncertainty did not exceed one degree on the instrument



scale. The advantage of using the extra filter for the bread eluate was less pronounced.

### Discussion

Though the choice of a low-grade flour and the use of concentrated extracts for some of the experimental work have exaggerated the difficulties, it is evident that there are many sources of error in the fluorometric procedure, as represented by the reference method, for the determination of riboflavin. Some of the most serious errors are a consequence of the limitations of Florisil as an adsorbent for riboflavin. These limitations vary from one lot of Florisil to another<sup>5</sup> and have a greater or lesser effect upon the assay results depending upon the concentration, volume, and clarity of the extracts from which the riboflavin is adsorbed. In a rapid method for the determination of riboflavin described by the authors, the adsorption step has been eliminated<sup>6</sup> (Hoffer, Alcock, and Geddes, 1944).

Since added riboflavin was more readily adsorbed by Florisil than the riboflavin native to flour extracts, good results of recovery tests can never justify confidence in the results of riboflavin assays. This does not mean that such tests are valueless, for low recoveries will certainly indicate low assay values. The test should be carried out, however, by adding the riboflavin before digestion and using the same weight of sample and the same aliquot volume as are used in the assay itself. The galvanometer reading must then be brought within the desired range by taking a suitable volume of eluate. The quality of a new lot of Florisil can best be checked by comparing it with satisfactory material. When this cannot be done, useful information as to the limitations of the Florisil may be secured by passing extracts through a number of adsorption tubes in series as described earlier.

Other practical consequences of these studies were that in later work no more than 1 g of sample was taken for assay, and, to be on the safe side, the amount of Florisil used per tube was increased from 1.1 to 1.8 g (6.25 cm).

Our experience also indicates the need for carrying out the adsorption from perfectly clear extracts. We have found a No. 40 Whatman paper suitable for filtering flour extracts and that filtration can be speeded up by use of Celite. This material does not adsorb riboflavin.

Pure riboflavin adsorbed from aqueous solution is very firmly held by Florisil and cannot be removed by washing with a large volume of

<sup>5</sup> The Floridin Company informed us that these variations have now been eliminated.

<sup>6</sup> While our studies were in progress, adsorption on Florisil was one of the steps in the fluorometric analyses of enriched flour and bread which was under investigation by the 1943-44 Subcommittee on Riboflavin Assay. The report of their studies (*Cereal Chem.* 21: 398-407, 1944) has appeared since the submission of this manuscript and confirms our finding that the adsorption step can be omitted.

water. Native riboflavin adsorbed from flour extracts, and possibly from other materials, is much less firmly held and some losses may occur when the Florisil is washed. Accordingly, the minimum amount of water (not more than 10 ml) should be used for washing.

### Summary

A number of sources of error in the fluorometric method for the determination of riboflavin have been studied.

Two factors may contribute to the sample size effect. They are: (1) Adsorption of riboflavin on the residues remaining after extraction. (2) Less complete adsorption by Florisil of the riboflavin from more concentrated extracts. The magnitude of the effect of the second factor depends upon the aliquot volume and the quality of the Florisil. Riboflavin adsorbed on the extraction residues goes into solution upon dilution.

Pure riboflavin is adsorbed more readily by Florisil than the native riboflavin of flour extracts. This difference must be taken into account when considering the significance of recovery tests. Suggestions have been made for carrying out recovery tests and for checking the adsorbing qualities of Florisil.

Extracts should be freed from any suspended material before they are passed over Florisil. Native riboflavin adsorbed on Florisil may be removed by washing, and accordingly the minimum amount of wash water should be used.

The use of Corning filter 511 alone for the incident light proved unsatisfactory owing to the photosensitivity of riboflavin in pyridine eluates and to the difficulty of finding the blank values for patent flour eluates. The use of a combination of Corning filters 511 and 038 for the incident light with 351 for the fluorescent light eliminated these difficulties as sources of important errors.

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1944 A rapid method for the determination of riboflavin in wheat and wheat products. *Cereal Chem.* 21: 524-533.

# A RAPID METHOD FOR THE DETERMINATION OF RIBOFLAVIN IN WHEAT AND WHEAT PRODUCTS<sup>1</sup>

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The need for a rapid precise assay of riboflavin in wheat and wheat products led to an investigation of the possibility of using a portion of the extract obtained in the determination of thiamine by the rapid method described by the authors (Hoffer, Alcock, and Geddes, 1943). From this starting point the rapid method of riboflavin assay described in this paper has been developed and employed in the analysis of wheats, flour and feed streams, and enriched flours in comparison with a reference procedure. The use of Florisil and pyridine is eliminated and a single determination can be made in 60 to 70 min.

## The Reference Method

The assay procedure employed as a standard of reference in these experiments was a modification of the fluorometric method used by the Subcommittee on Riboflavin Assay (Andrews, 1943, 1943a). The essential features of this reference method have been described by Hoffer, Alcock, and Geddes (1944) in connection with their study of certain steps in the fluorometric method. In view of the results of the experiments on light filters, all the reference method values given in the present paper were obtained by using a combination of Corning glass filters 511 and 038 for the incident light and 351 for fluorescent light.

## The Rapid Method

After some preliminary studies the following rapid method for the determination of riboflavin in wheat and wheat products was developed.

### *Apparatus*

- (1) Photofluorometer.
- (2) Centrifuge and 50-ml centrifuge tubes, preferably without lip.
- (3) Boiling tubes.

### *Reagents*

- (1) Potassium chloride solution. Dissolve 250 g in 2% acetic acid and make up to 1 liter.
- (2) Potassium permanganate solution. 0.5% freshly prepared.
- (3) Hydrogen peroxide 0.3%.
- (4) Sodium fluorescein. Stock solution—10 mg to 1 liter. Working standard (0.05  $\mu\text{g/ml}$ )—dilute stock solution 5 to 1000.
- (5) Sodium hydrosulfite.
- (6) Riboflavin standard. 1  $\mu\text{g/ml}$ .

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*Method.* Place 0.5 g of sample in a 50-ml centrifuge tube with 20 ml of the KCl solution. Break up any lumps by means of a glass rod. Place the centrifuge tube in a water bath at 70°C, cover with a boiling tube to prevent evaporation, and leave for 30 min. At the end of this time, stir the mixture to break up any lumps that have formed, and then centrifuge at 2500 rpm for at least 10 min.

Transfer a 5-ml aliquot of the supernatant solution to a cuvette and add 5 ml of water. Now add rapidly 1 ml of  $\text{KMnO}_4$  solution and, after  $\frac{1}{2}$  min, 1 ml of  $\text{H}_2\text{O}_2$ . After the gas bubbles have cleared, read the fluorescence (A). Add 1 ml of riboflavin standard and again read the fluorescence (B). Add about 30 or 40 mg of solid sodium hydrosulfite, followed by further additions of about 10 mg each, and read the fluorescence after each addition. When an addition of hydrosulfite fails to reduce the reading, that reading is taken as the blank fluorescence (C).

The riboflavin content of the sample is calculated as follows:

$$\text{Riboflavin } \mu\text{g/g} = \frac{A - 1.08C}{1.08B - A} \times 8.$$

The use of the factor 1.08 is necessitated by the increase in the volume of the liquid in the cuvette from 12 ml to 13 ml upon the addition of the riboflavin standard.

*Sample Size Effect in the Rapid Method.* That sample size affects assay values obtained by the rapid method is evident from the results in Table I.

TABLE I  
EFFECT OF SAMPLE SIZE ON THE RESULTS OF ASSAYS FOR RIBOFLAVIN  
BY THE RAPID METHOD

Weight of sample	Riboflavin content					
	Patent flour	Enriched flour	Enriched bread	Whole wheat	Bran	Shorts
g	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
1.50	0.20	2.15	2.70	1.04	2.39	2.47
1.00	0.22	2.10	2.77	1.06	2.57	2.85
0.75	0.26	2.31	2.82	1.07	2.87	2.99
0.50	0.29	2.44	3.09	1.16	2.98	3.17

In order to minimize the effect of sample size, the weight of sample taken for assay by the rapid method was fixed at 0.5 g. It was impracticable to use a smaller quantity because of the low fluorescence readings of the extracts.

*Filters.* The Corning 351 filters supplied with the Coleman photo-fluorometer vary considerably in density. With the same solution of sodium fluorescein and the same setting of the diaphragm, three filters gave the following instrument readings: 60, 49, and 40. All the work reported here was done with the least dense of these three filters and even then the readings for unenriched patent flours were very low.

For the purpose of increasing the fluorescence readings, filter 038 was removed when dealing with rapid method extracts. No difficulty was experienced in getting satisfactory blank readings without this filter, as will be apparent from Table II which shows the effect of additions of sodium hydrosulfite on the fluorescence of rapid method extracts.

TABLE II

EFFECT OF ADDITIONS OF SODIUM HYDROSULFITE ON THE FLUORESCENCE READINGS OF RAPID METHOD EXTRACTS—WITHOUT FILTER C. G. 038

Hydrosulfite added	Fluorescence readings <sup>1</sup>		
	Flour	Bread	Bran
<i>mg</i>			
10	1.5	3.5	15.0
20	1.5	2.8	8.6
30	1.3	2.5	6.1
40	1.0	2.4	5.1
50	—	2.3	5.2
60	—	2.3	4.7
70	—	2.5	4.7
80	—	—	4.5
90	—	—	4.3
100	1.0	—	4.3
Estimated blank	1.0	2.5	4.5–5.0

<sup>1</sup> Units of deflection of Coleman photofluorometer corrected for dilution. Ordinarily the instrument is read to the nearest 0.5°.

Rapid method extracts have a lower fluorescence and are much less sensitive to ultraviolet light than pyridine eluates, even though 25% potassium chloride in 2% acetic acid transmits 99% of the light transmitted by water. Because of this greater stability, riboflavin assays by the rapid method can be made in diffused daylight. When using the reference method, the blinds were drawn, the laboratory was illuminated with orange-colored lights, and solutions containing riboflavin were covered with a black cloth.

It should be noted that 2 or 3 min after the first addition of sodium hydrosulfite to a rapid method extract, the solution starts to become opalescent owing to the precipitation of sulfur. There is, however, plenty of time before this occurs to determine the blank. In the case of pyridine eluates, the released sulfur is dissolved, causing the solution to turn yellow.

*Concentration of Potassium Chloride for Extraction.* Because 25% potassium chloride in 2% acetic acid was used as the extractant in the rapid method for thiamine assay, it was also used for the riboflavin determination. But when it was decided to fix the sample weight at 0.5 g for riboflavin assays, and it became no longer possible to consider the routine use of portions of the same extract for both thiamine and riboflavin determinations, no strong *a priori* reason for using this particular extractant remained.

In the method as outlined above, 5 ml of extract is diluted with 5 ml of water before oxidation with permanganate. Before trying other concentrations of potassium chloride, a few determinations were made

TABLE III  
RIBOFLAVIN VALUES AS DETERMINED BY MEASURING THE FLUORESCENCE OF  
UNDILUTED AND DILUTED RAPID METHOD EXTRACTS AND BY  
THE REFERENCE METHOD

Sample	Riboflavin content		
	Rapid method		Reference method
	Undiluted extract	Diluted extract	
	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
Bread	3.44	3.21	3.34
Bran	4.23	3.13	3.23
Shorts	3.76	2.91	2.60
Whole wheat	1.39	1.21	1.21

using 10 ml of the undiluted extract for fluorometric measurement. The results are given in Table III.

The diluted extract gave results closer to those obtained by the reference method than did the undiluted extract. Another disadvantage of the undiluted extract was that it foamed excessively when hydrogen peroxide was added and took a longer time to clear.

Extractions with various concentrations of potassium chloride in 2% acetic acid gave the results reported in Table IV. In each case 5 ml of the extract was diluted with 5 ml of water before the addition of permanganate.

TABLE IV  
RIBOFLAVIN VALUES AS DETERMINED BY EXTRACTION WITH  
VARIOUS CONCENTRATIONS OF POTASSIUM CHLORIDE

KCl concentration	Riboflavin content						Fluorescence reading <sup>1</sup> —1 $\mu\text{g}$ of riboflavin
	Flour	Bread	Bran	Shorts	Whole wheat	Germ	
%	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	
0	—	3.21	2.90	3.20	1.32	4.47	71
5	0.32	3.35	2.64	3.59	1.12	4.48	60
10	0.34	3.13	3.24	3.00	1.13	5.08	52
15	0.34	3.27	3.29	2.80	1.24	5.04	48
20	0.37	3.16	3.24	2.87	1.25	5.06	45
25	0.34	3.21	3.13	2.91	1.21	5.28	42
Reference method	0.32	3.34	3.24	2.60	1.21	5.03	—

<sup>1</sup> Obtained by adding 5 ml of water, 1 ml of  $\text{KmnO}_4$  solution, 1 ml of  $\text{H}_2\text{O}_2$  solution, and 1 ml of the riboflavin standard to 5 ml of KCl solution of the indicated concentration.

Concentrations of 0% and 5% were ruled out because they yielded cloudy extracts in the case of flour, shorts, and whole wheat. There was little to choose between the other concentrations of potassium

chloride and, as some data had already been accumulated using the 25% solution, it was employed for the rest of the work.

### Application of the Rapid Method to Flours and Mill Feeds

All the flour and feed streams from a large commercial mill were analyzed for riboflavin, in duplicate and on different days, by the rapid

TABLE V

RIBOFLAVIN VALUES ON FLOUR AND FEED STREAMS AS DETERMINED BY THE REFERENCE METHOD AND BY THE RAPID METHOD WITH AND WITHOUT PERMANGANATE TREATMENT

Stream	Ash	Riboflavin content		
		Reference method	Rapid method	
			With $\text{KMnO}_4$	Without $\text{KMnO}_4$
	%	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
(a) 2nd middlings	0.34	0.34	0.35	0.36
4th middlings	0.37	0.42	0.33	0.41
1st middlings	0.38	0.34	0.35	0.46
3rd middlings	0.39	0.47	0.36	0.41
Coarse sizer	0.41	0.44	0.31	0.34
Fine sizer	0.43	0.38	0.39	0.47
5th middlings	0.44	0.44	0.36	0.39
6th middlings	0.48	0.41	0.40	0.51
7th middlings	0.49	0.40	0.37	0.56
2nd and 3rd break	0.51	0.49	0.38	0.53
1st tailings	0.54	0.55	0.43	0.61
Roll dust	0.55	0.47	0.46	0.54
8th middlings	0.61	0.55	0.49	0.72
4th break	0.68	0.41	0.43	0.60
2nd quality	0.72	0.59	0.56	0.67
Purifier dust	0.72	0.57	0.49	0.63
1st break	0.72	0.59	0.56	0.70
1st middlings scalp	0.75	0.53	0.55	0.67
1st chip	0.84	0.69	0.67	0.80
1st germ	0.93	0.69	0.66	0.87
1st low grade	0.96	0.79	0.69	0.86
5th break	1.12	0.85	0.81	1.06
2nd germ and chip	1.28	0.99	0.88	1.18
2nd low grade	1.37	1.01	0.95	1.13
Mean—all flour streams		0.56	0.51	0.65
(b) Low grade tailings	1.78	1.06	0.91	1.36
3rd low grade	2.04	1.66	1.60	1.95
Bran and shorts duster redresser	2.25	1.61	1.55	2.23
Bran and shorts reel	2.30	1.68	1.50	2.05
Feed middlings	2.90	2.52	2.62	3.91
Feed middlings	3.31	2.37	2.30	3.38
Feed middlings	3.75	3.34	3.31	4.89
Shorts	4.19	2.99	3.18	5.53
Bran	5.76	2.56	2.65	4.28
Mean—all feed streams		2.20	2.18	3.28
Mean—for all samples		1.01	0.97	1.36

method with and without permanganate treatment. The feed samples were analyzed in duplicate on different days by the reference method, but the reference method values for the flour samples were the results of single determinations. The results are given in Table V.

Generally speaking, there was excellent agreement between the values obtained by the rapid method using permanganate and by the reference method. The results of a statistical analysis of the values in Table V are given in Table VI.

TABLE VI

MEANS, STANDARD DEVIATIONS, AND CORRELATION COEFFICIENTS OF RIBOFLAVIN VALUES AS DETERMINED BY THE REFERENCE METHOD AND THE RAPID METHOD (Using permanganate)<sup>1</sup>

Material	Mean		Standard deviation		Correlation coefficient
	Reference	Rapid	Reference	Rapid	
Flour streams	0.56	0.51	0.19	0.18	0.970
Feed streams	2.20	2.18	0.70	0.78	0.994
All streams	1.01	0.97	0.83	0.86	0.997

<sup>1</sup> Statistical summary of data in Table V.

The results of assays by the rapid method were slightly lower on the average than those obtained by the reference method. Although this difference is statistically significant it is so small that for practical purposes it can be ignored.

The rapid method assays without permanganate were carried out to determine whether oxidation of the extract was essential. It will be seen from Table VII that the effect of permanganate treatment increased as the ash content of the material increased.

For lower grade flours and feeds, treatment of rapid method extracts with permanganate is obviously necessary, but with flours containing up to 0.60% ash, the results were equally good whether permanganate was used or not. For enriched and unenriched patent flours, oxidation with permanganate can therefore be omitted, although this step was carried out in securing the rapid method results still to be considered.

#### Application of the Rapid Method to Whole Wheat

Sixteen samples of whole wheat, ground so that all except a small amount of branny material passed a 44 GG sieve, were analyzed in duplicate on different days by both methods. The means of the results were as follows:

Reference method	1.12 $\mu\text{g/g}$
Rapid method	1.08 $\mu\text{g/g}$



TABLE VII

MEAN RIBOFLAVIN VALUES FOR MILL STREAMS GROUPED ACCORDING TO ASH CONTENT; AS DETERMINED BY THE REFERENCE METHOD AND THE RAPID METHOD WITH AND WITHOUT PERMANGANATE

No. of samples	Ash range	Riboflavin content		
		Reference method	Rapid method	
			With $\text{KMnO}_4$	Without $\text{KMnO}_4$
	%	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
4	0.35-0.39	0.39	0.35	0.41
5	0.40-0.49	0.41	0.37	0.45
3	0.50-0.59	0.50	0.44	0.56
6	0.60-0.79	0.54	0.51	0.67
3	0.80-0.99	0.72	0.67	0.84
4	1.00-1.99	0.98	0.89	1.18
4	2.00-2.99	1.87	1.82	2.54
2	3.00-3.99	2.86	2.81	5.21
2	4.00-5.99	2.78	2.92	3.79

As was the case with the mill streams, the rapid method gave results that, on the average, were  $0.04 \mu\text{g/g}$  lower than those yielded by the reference method. Though small this difference was significant.

### Precision of the Reference and Rapid Methods

The standard errors and coefficients of variability for different materials are shown in Table VIII.

TABLE VIII

STANDARD ERRORS AND COEFFICIENTS OF VARIABILITY OF THE REFERENCE METHOD AND THE RAPID METHOD

Material	Method	Number of samples	Mean riboflavin content	Standard error <sup>1</sup> Single determination	Coefficient of variability
			$\mu\text{g/g}$	$\mu\text{g/g}$	%
Flour <sup>2</sup>	Rapid	24	0.50	0.04	8.0
Mill feed	Reference	9	2.20	0.29	13.1
	Rapid	13	2.36	0.13	5.5
Wheat	Reference	16	1.12	0.07	6.3
	Rapid	16	1.08	0.06	5.6
Wheat kernels glumes and stems <sup>3</sup>	Reference	29	1.54	0.14	8.8
	Reference	56	3.17	0.23	7.1
	Reference	45	4.98	0.36	7.2
	Reference	57	6.94	0.44	6.4
	Reference	9	8.70	0.44	5.0

<sup>1</sup> From results of duplicate determinations on different days.

<sup>2</sup> Duplicate determinations on different days were made by the reference method on these 24 samples of flour using 5 g of sample. The mean riboflavin content was  $0.47 \mu\text{g/g}$ , the standard error of a single determination  $0.07 \mu\text{g/g}$ , and the coefficient of variability 14.9%. Single determinations by the reference method using 1 g samples gave a mean value of  $0.56 \mu\text{g/g}$ .

<sup>3</sup> Samples at different stages of development obtained in connection with another study.

For both methods the standard error in absolute terms increased with the riboflavin content, but on a percentage basis it decreased with increasing riboflavin content. As might be expected in view of its greater simplicity, the error for the rapid method was lower than for the reference method. Although the percentage error for the rapid method may still appear to be large, especially in the case of flours, the absolute error was within what may be regarded as acceptable limits for a determination of this nature.

Riboflavin values for several collaborative check samples of enriched flour and bread have been determined by the reference and rapid methods. The results are given in Table IX together with the mean, minimum, and maximum values for the collaborators. The authors' data on an additional series of wheat flours which had been analyzed in another laboratory are given in Table X. These comparative values

TABLE IX  
A COMPARISON OF RIBOFLAVIN VALUES FOR CHECK SAMPLES

A.A.C.C. collaborative samples	Riboflavin content					
	Collaborative values		Authors' values			
			Reference method		Rapid method	
	Mean	Range	Mean	Range	Mean	Range
	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
April						
1943 enriched flour	2.98	2.29-4.00	2.93	2.78-3.07	2.83	2.73-2.99
October						
1943 enriched flour	3.15	2.20-4.39	2.75	2.50-2.86	3.01	2.96-3.09
December						
1943 enriched flour	2.62	1.48-3.39	3.12	2.86-3.47	2.94	2.90-2.98
February						
1944 enriched bread	2.60	1.54-3.44	2.92	2.85-3.00	2.69	2.65-2.84
Mean	2.84		2.93		2.87	

TABLE X  
A COMPARISON OF RIBOFLAVIN VALUES FOR SAMPLES SUPPLIED BY J. S. ANDREWS<sup>1</sup>

Material	Andrews' values		Authors' values	
	Micro-biological	Fluoro-metric	Reference method	Rapid method
Patent flour	0.36	0.32	0.28	0.28
Enriched patent	2.60	2.58	2.49	2.45
Enriched bread	2.66	2.95	3.34	3.03
Whole wheat flour	1.11	1.13	1.14	1.21
Mean	1.68	1.75	1.81	1.74

<sup>1</sup> General Mills Inc., Research Dept., Minneapolis, Minnesota.

show that the authors' results are in good agreement with those obtained by other laboratories.

### Discussion

The elimination of adsorption on Florisil, with all the hazards attending this step, is the chief advantage of the rapid method.<sup>5</sup>

The use of the rapid method not only reduces the duplicate error but also results in a considerable saving of time. A single determination by the reference method takes 2½ to 3 hr and by the rapid method not more than 70 min. A patent flour, enriched or unenriched, can be easily assayed in 1 hr, since in this case it is unnecessary to oxidize the extract with permanganate. Another advantage of the rapid method is the avoidance of the use of pyridine.

The low fluorescence of extracts of unenriched patent flours constitutes the chief drawback of the rapid method. A flour containing 0.4 µg/g of riboflavin yields an extract with a fluorescence reading of 3 and a blank of 1 on our Coleman photofluorometer.

Even when the reference method is used for assaying unenriched patent flours, some special difficulties are encountered owing to their low riboflavin content, for, in order to get a satisfactory concentration of riboflavin in the eluates, one must run the risks of error associated with the use of larger samples or larger aliquots. However, on account of their low and comparatively uniform riboflavin content, the analyst is perhaps least interested in unenriched patent flours, though the fact that they can be satisfactorily assayed by the rapid method, in spite of the low fluorescence of their extracts, is made evident by the data which have been presented.

With enriched patents, the rapid method readings are much better. A sample containing 3.0 µg/g gives a reading of approximately 18 with a blank of 2. The rapid method also works well with enriched bread, whole wheat, wheat germ, and mill feeds, while a few tests have indicated that it can be adapted for assaying dry and liquid milk. It is unsuitable for assays on green plant tissue.

### Summary

A rapid and relatively simple method for the assay of riboflavin in wheat and wheat products has been described. It eliminates adsorption on Florisil and the use of pyridine. A single determination can be made in 60 to 70 min.

<sup>5</sup> During the course of our studies the effect of Florisil and permanganate in the fluorometric analysis of enriched flour and bread was under investigation by the 1943-44 Subcommittee on Riboflavin Assay. The report of these studies (Cereal Chem. 21: 398-407, 1944), which appeared since the submission of this manuscript, showed that the use of Florisil resulted in poor agreement between laboratories and indicated that relatively simple assay methods could be employed. With enriched flours direct reading of flour extracts gave just as satisfactory a result as when either Florisil or permanganate was employed. In assaying enriched bread, treatment of the extract with Florisil had no effect on the final results but the use of permanganate aided in removing interfering impurities.

Results of assays of flour, mill feeds, and bread by the rapid method agreed closely with those obtained by a modification of the regular fluorometric procedure employed as a standard of reference.

For both the rapid and reference methods, the absolute error increased, while the percentage error decreased, with increasing riboflavin content. The duplicate error for the rapid method was lower than for the regular method.

The rapid method works well with enriched flour and bread, mill feeds, and wheat, and there are indications that it can be applied to other materials, though not to green plant tissues. In spite of low fluorescence readings it is capable of giving satisfactory results for unenriched patent flours.

#### Acknowledgment

The authors wish to acknowledge their indebtedness to J. S. Andrews, General Mills Inc., Research Department, Minneapolis, Minnesota, for furnishing four samples with information as to their riboflavin content.

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## A MODIFICATION OF THE KNEEN AND SANDSTEDT METHODS FOR THE DETERMINATION OF ALPHA- AND BETA-AMYLASES IN BARLEY MALT<sup>1</sup>

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Methods for the determination of alpha- and beta-amylase in malt have been developed by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941). The methods were designed as a measure of alpha-dextrinizing, malt (alpha- + beta-) saccharifying activities, and, by difference, the beta-saccharifying activity of malt. There seemed no objection to adapting the determination of alpha-dextrinizing activ-

<sup>1</sup> Cooperative investigations between the University of Wisconsin and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

ity to 20°C and substituting the regular diastatic procedure for the malt-saccharifying activity determination proposed by these workers (1941). This change would make the method more conveniently applicable in malt laboratories using the diastatic power procedure. This modification has involved the establishment of the relation between saccharification and dextrinization of alpha-amylase under the modified experimental conditions. Another method of preparation of enzymatically pure beta-amylase has been tested and found to be satisfactory in the preparation of the standard alpha-amylodextrin substrate.<sup>2</sup> A new unit is proposed for alpha-amylase based upon the diastatic power unit in maltose equivalent.<sup>3</sup>

### Preparation of Amylase Components

It was essential that enzymatically pure preparations of both alpha- and beta-amylase be made. The alpha-amylase preparations were used in the establishment of the relation existing between alpha-saccharifying and alpha-dextrinizing activities, and the beta-amylase in the preparation of the alpha-amylodextrin substrates.

*Malt Alpha-Amylase.* The method of Ohlsson as modified by Kneen, Sandstedt, and Hollenbeck (1943) was used in the preparation of malt alpha-amylase.

*Barley Beta-Amylase.* Two methods have been used for the preparation of barley beta-amylase. The first is that described by Kneen, Sandstedt, and Hollenbeck (1943). The second is a modification of the fractional alcohol precipitation technique used by Meyer, Bernfeld, and Press (1940) for wheat. Briefly, the latter method involves: (1) concentration of a 1 : 5 aqueous barley extract to one third its volume under reduced pressure, (2) making the concentrated extract up to an ethanol concentration of 65% in the cold, separating and discarding the material settling out, and (3) raising the ethanol concentration of the clear supernatant to 80% ethanol in the cold, and isolation of the separated material.

Beta-amylase preparations made by the technique of Kneen, Sandstedt, and Hollenbeck (1943) were less soluble in water than those prepared by the fractional alcohol precipitation technique but were equally satisfactory when the test described later in this paper was applied to them. While considerable individual variation was apparent, the saccharifying activities of preparations made by both techniques were of the same order.

<sup>2</sup> Alpha-amylodextrin is the limit dextrin produced by the action of beta-amylase on soluble starch.  
<sup>3</sup> "Maltose equivalent" is the unit of diastatic power preferred to the older "degrees Lintner" by the American Society of Brewing Chemists. Maltose equivalent is defined as "the grams reducing substances expressed as the grams maltose produced from 100 g malt acting on soluble starch under the specified experimental conditions." "Degrees Lintner" is obtained by dividing the maltose equivalent by 4.

### Test for Purity of Beta-Amylase

A test for the absence of alpha-amylase in the beta-amylase is necessary when the beta-amylase is to be used in the preparation of the standard amylo-dextrin substrate. The proposed test is simple in principle, and has been found to be sensitive to small contamination by alpha-amylase. The test also serves to fix the minimal quantity of beta-amylase essential for complete conversion of the starch to amylo-dextrin in 24 hr.

To 50 ml portions of 4% soluble starch (special for diastatic power) in 100 ml volumetric flasks are added increasing amounts of the beta-amylase preparation dissolved in 5 ml buffer mixture (Sandstedt, Kneen, and Blish; 1939) with or without additional water. Obviously, the range of additions of beta-amylase to the starch is determined by the activity of the preparation tested. A six-fold range in concentration has been found satisfactory. The volumetric flasks are made to the mark with water, toluene added, the contents mixed well, and allowed to stand 24 hr at room temperature.

After 24 hr, dextrinization measurements are made upon the various substrates by the method of Sandstedt, Kneen, and Blish as modified in this paper, using a standard source of malt alpha-amylase as dextrinizing agent. A constant dextrinizing time over a range of beta-amylase concentration is indicative of freedom from alpha-amylase contamination. The use of amylase preparations of bacterial, fungal, or animal origin instead of malt alpha-amylase is not recommended, as the application of the method to these enzymes has not been established. In the preparation of the standard amylo-dextrin substrate, the lowest concentration at which a constant dextrinizing time is attained in the test above, plus 25% excess, has been used.

### Relation between Saccharifying and Dextrinizing Activities of Alpha-Amylase

In order to correct for the saccharification by alpha-amylase in the diastatic power determination, it is essential that the relation between the dextrinizing and saccharifying activities of alpha-amylase be established. By use of this relationship, the saccharification by beta-amylase can be calculated accurately and expressed as the difference between the malt and alpha-amylase saccharification.

The saccharification by various quantities of alpha-amylase was carried out in duplicate by use of the diastatic power procedure (Cereal Laboratory Methods; 4th ed., 1941). Dextrinization measurements of the same quantities of alpha-amylase used in the saccharification measurements were made by the method of Sandstedt, Kneen, and

Blish (1939) as modified herein. These dextrinization values were obtained in duplicate on each of two substrates. The substrates were made with two different beta-amylase concentrates, prepared by each of the two methods mentioned above. Agreement between the four dextrinization determinations was very good, if dextrinization times below 10 min were avoided.

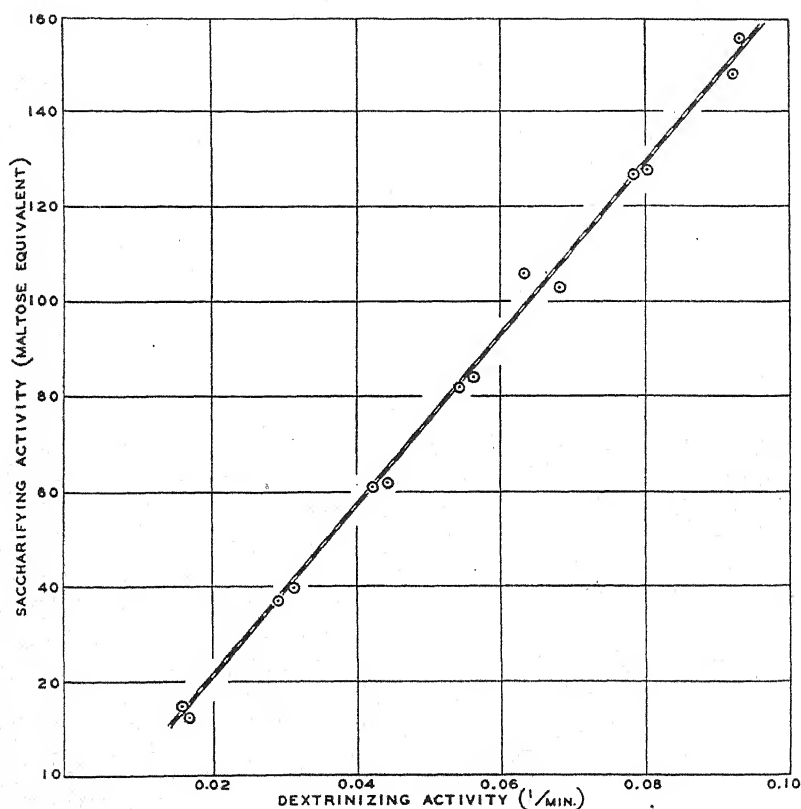


Fig. 1. The relation between alpha-amylase dextrinizing and saccharifying activities.

The saccharification values were calculated by multiplying the ml potassium ferricyanide reduced by 144, the factor used in calculating the diastatic power (maltose equivalent), when 1 ml (0.05 g malt equivalent) diastatic extract is used in the diastasis. The relation between the average saccharification values expressed in maltose equivalent and the dextrinizing activity expressed as reciprocal minutes is shown in Figure 1. A series of values was read from the graph, and is given in Table I.

TABLE I  
SACCHARIFICATION EQUIVALENTS "K" (MALTOSE EQUIVALENT) OF DIFFERENT  
QUANTITIES OF ALPHA-AMYLASE, EXPRESSED IN RECIPROCAL MINUTES

Dextrinization 1/min	Saccharification equivalent "K"	Dextrinization 1/min	Saccharification equivalent "K"
0.020	21	0.065	103
0.025	30	0.070	111
0.030	39	0.075	121
0.035	48	0.080	130
0.040	57	0.085	139
0.045	66	0.090	148
0.050	75	0.095	157
0.055	84	0.100	165
0.060	93		

### The Proposed Methods for Alpha- and Beta-Amylase in Malt

The reagents and procedure are the same as given by Sandstedt, Kneen, and Blish (1939) for the determination of alpha-amylase except that (1) 20°C instead of 30°C was used for the reaction, and (2) extraction of the malt was carried out according to the Cereal Laboratory Methods (4th ed., 1941) diastatic power procedure. For the determination of malt-saccharifying activity, the diastatic power procedure (Cereal Laboratory Methods; 4th ed., 1941) is followed exactly as published.

Minor changes in the method of color comparison are being used successfully in the Barley and Malt Laboratory at Madison. An inorganic color standard suggested by Landis<sup>4</sup> was modified slightly and substituted for the dextrin standard described by Sandstedt, Kneen, and Blish. This standard is prepared as follows: 25 g  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  and 3.84 g  $\text{K}_2\text{Cr}_2\text{O}_7$  per 100 ml 0.01*N* HCl.

Color comparisons were made visually through 6.0–6.2 cm (6 ml) of the iodine-digest complex in 4-inch test tubes set over a 15 watt day-light fluorescent bulb against 6.0 ml of the color standard described above. In using this procedure it is essential that a set of test tubes be obtained varying by not more than 2 mm in the depth of 6 ml of the liquid.

### Calculations

It has been shown by Kneen and Sandstedt (1941) and by the writers (unpublished) that the effects of alpha- and beta-amylase are additive in saccharification, even at relatively high ratios of beta- to alpha-amylase. This being the case, it is possible to determine accurately the alpha-amylase by suitable dextrinization measurements and apply a correction for its action on soluble starch in the malt saccharification (diastatic power) determination. That is, the dif-

<sup>4</sup>Presented in a paper given by Quick Landis at the 1943 Annual Meeting of the A.A.C.C.



ference between the diastatic power and the alpha-amylase saccharifying activity obtained by use of Table I is equal to the beta-amylase saccharifying activity.

*Malt Saccharifying Activity (diastatic power)*: The malt saccharifying activity (diastatic power) is expressed in terms of maltose equivalent as follows:

$$\text{D.P. [maltose equivalent (dry basis)]} = \frac{(\text{blank-titration}) \times 144 \times 100}{(100 - \% \text{ moisture})}$$

when 1 ml (0.05 g malt equiv.) extract is used in saccharification and 5 ml digest is used in the reducing power determination.

*Alpha-amylase Saccharifying Activity*: The saccharification due to alpha-amylase in the diastatic extract is given by

$$A = \frac{K \times 100}{(100 - \% \text{ moisture}) \times \text{ml diastatic extract used in dextrinization}}$$

where

A = alpha-amylase saccharifying activity (maltose equivalent, dry basis)

and

K = the saccharification equivalent (maltose equivalent) of alpha-amylase dextrinization (reciprocal minutes). The values for, "K" are obtained from Table I.

*Beta-Amylase Activity*: The difference between the malt-saccharifying activity, and the alpha-amylase saccharifying activity is equal to the beta-amylase activity.

$$\text{i.e., } B = \text{D.P.} - A$$

where

B = the beta-amylase activity [maltose equivalent (dry basis)].

A sample calculation using the above methods follows:

0.05N potassium ferricyanide reduced by 5 ml	
digest when 1 ml (0.05 g malt equivalent) diastatic extract is used in diastasis	= 5.00 ml
Moisture in malt sample	= 5.0%
Diastatic power = $\frac{5.00 \times 144 \times 100}{95.0}$	= 758 maltose equivalents
ml diastatic extract used in dextrinization	= 2 ml
Dextrinizing time (min to end point)	= 20 min
1/dextrinizing time = (1/20)	= 0.050

"K" value equivalent to 0.050 recip. min (Table I) = 75

Alpha-amylase saccharification =  $\frac{75 \times 100}{95 \times 2}$  = 40 maltose equivalents

Beta-amylase activity = 758 - 40 = 718 maltose equivalents

This hypothetical malt would have an alpha-amylase activity of 40 maltose equivalents, beta-amylase activity of 718 maltose equivalents, and diastatic power of the sum of the two, or 758 maltose equivalents.

### Summary

The original method of Sandstedt, Kneen, and Blish for alpha-amylase has been modified to make it more applicable to routine determinations in barley malt laboratories.

The dextrinization is measured at 20° instead of 30°C and the A.A.C.C. (Cereal Laboratory Methods) diastatic power procedure is substituted for the malt saccharifying activity determination of Kneen and Sandstedt.

The relation between alpha-amylase saccharifying and alpha-amylase dextrinizing activity has been established under these modified experimental conditions. By the use of the values so obtained, suitable corrections for alpha-amylase saccharifying activity can be made, and a valid measure of beta-amylase activity secured.

Two methods of preparation of enzymatically pure beta-amylase have been tested, and a simple method for ascertaining the presence of alpha-amylase described.

### Acknowledgments

The encouragement and cooperation of Eric Kneen, members of the Malt Analysis Committee of the American Association of Cereal Chemists, and members of the Diastatic Power Committee of the American Society of Brewing Chemists, and others have aided greatly in the completion of the work reported here. Mrs. G. B. Alexander cooperated in the necessary laboratory work.

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# A STUDY OF SOME OF THE VITAMIN B-COMPLEX FACTORS IN RICE AND ITS MILLED PRODUCTS, II

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The riboflavin and biotin content of different varieties and milled fractions of rice have been investigated and the findings reported herein are complementary to the study of thiamine, nicotinic acid, pantothenic acid, and pyridoxine presented in an earlier account of these experiments (Williams, Knox, and Fieger, 1943).

Although the riboflavin content of a similar group of rice samples was investigated by Kik (1943), few figures on the biotin content of rice products have appeared. Cheldelin and Williams (1942), in their report on the B vitamin content of various foods, supplied data for only one rice product, a commercially processed and packaged breakfast cereal. Lampen, Bahler, and Peterson (1942) reported the biotin content of one sample of rice polish and one sample of a rice bran concentrate. Cheldelin, Woods, and Williams (1943) include findings for one sample of polished rice in their investigation of B vitamin losses upon cooking various foods.

## Materials and Methods

The samples used for these experiments were the same ones analyzed in the first part of the study, already mentioned. They were divided into two main groups: (1) samples of brown rice of six typical Louisiana varieties (Blue Rose, Fortuna, Early Prolific, Nira, Rexoro, and American Pearl) collected to study differences among varieties; and (2) samples of milled fractions of three typical varieties (Blue Rose, Early Prolific, and Fortuna) collected to investigate differences in vitamin content of the various milled fractions.

As pointed out in the earlier publication, these milled fractions consist of the products obtained in the different steps of the milling process. As more and more of the outer coats of the rice grain are removed and the rice progresses from the original rough rice to the finished product, seven fractions are obtained: (1) brown rice, (2) first-break rice, (3) second-break rice, (4) brushed rice, and (5) finished rice. The outer coats of the grain appear at the end of the process as (6) rice polish and (7) rice bran.

The samples were analyzed by the following procedures:

1. Riboflavin—the Conner and Straub (1941) fluorometric method.
2. Biotin—the Shull, Hutchings, and Peterson (1942) microbiological assay procedure for biotin, as modified by Shull and Peterson (1943). The samples were hydrolyzed in normal sulfuric acid at 15 lb of pressure for 30 min.

### Discussion of Results

*Riboflavin.* Remarkably close agreement was found between the figures given in Table I and those reported by Kik (1943). In many

TABLE I

COMPARISON OF THE RIBOFLAVIN AND BIOTIN CONTENT OF THE MILLED FRACTIONS OF SEVERAL VARIETIES OF RICE

Variety	Number of samples brown rice	Number of samples each other fraction	Milled fractions <sup>1</sup>						
			Brown rice	1st break rice	2nd break rice	Brushed rice	Fin-ished rice	Bran	Polish
			μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
RIBOFLAVIN									
Blue Rose	5	3	0.76	0.48	0.49	0.40	0.29	2.12	1.65
Fortuna	7	3	0.52	0.45	0.29	0.25	0.23	1.83	1.92
Early Prolific	5	3	0.56	0.34	0.20	0.18	0.20	2.48	1.77
Average			0.61	0.42	0.33	0.28	0.24	2.14	1.78
BIOTIN									
Blue Rose	5	3	0.114	0.061	0.059	0.053	0.050	0.541	0.543
Fortuna	7	3	0.123	0.056	0.050	0.051	0.034	0.485	0.833
Early Prolific	5	3	0.126	0.082	0.059	0.048	0.043	0.377	0.591
Average			0.121	0.066	0.056	0.051	0.042	0.468	0.656

<sup>1</sup> Measurements are for dry weight.

cases, values for the same milled fraction were within 5 to 10% of each other. In both cases, the fluorometric method of analysis was used.

The decrease in vitamin content with the increase in milling was illustrated by the riboflavin values, just as it has been by the data published in 1943. The sharpest drop in riboflavin content occurred between the brown rice and first break.

When compared with other foods, brown rice, polish, and bran do not rank very high in riboflavin content. White and yellow corn contain more riboflavin per unit weight than does brown rice, while wheat germ and wheat bran considerably exceed rice polish and rice bran in riboflavin content (Conner and Straub, 1941).

*Biotin.* Some difficulty was experienced with the biotin assay. The first hindrance was encountered in the preparation of the yeast filtrate, although the Shull and Peterson (1943) modified procedure was followed. It was found that warming the various solutions containing norit by means of a controlled bath alone did not produce a heat penetration sufficiently rapid for successful adsorption or elution, as the case might be. Heating to 55–60°C by means of a hot plate prior to placing the solution in a water bath, however, provided sufficient heat penetration and produced a highly satisfactory yeast filtrate.

A second difficulty was encountered as a result of anomalous values obtained in the assays of rice polish, rice bran, and brown rice. Similar drifts have been observed in the assays of cereal products as reported by Strong and Carpenter (1942) and Neal and Strong (1943). The effect produced most markedly by rice polish is probably one of stimulation as well as one of inhibition. The drift effect was obviated by reading samples at dilutions giving agreement within 10% for assays dosed at increasing levels of concentration. An assay of defatted rice polish indicated that the interfering substance was not in the fat fraction. Further work is being continued on this phase of the problem.

Although the biotin values reported in the present study were somewhat higher than any for rice samples published by Lampen, Bahler, and Peterson (1942), Cheldelin and Williams (1942), or Cheldelin, Woods, and Williams (1943), it should be pointed out that these workers reported values for only one sample in each case. Average values on the air-dry basis obtained in the present work, furthermore, agreed within 5% for brown rice and within 2% for rice polish with values obtained by workers at the Department of Biochemistry, University of Wisconsin, for brown rice and rice polish samples furnished by this laboratory. All samples here reported were checked against a control supplied by that laboratory.

TABLE II  
COMPARISON OF THE RIBOFLAVIN AND BIOTIN CONTENT OF SEVERAL  
VARIETIES OF BROWN RICE

Variety	Number of samples	Vitamin content	
		Riboflavin	Biotin
		$\mu\text{g/g dry wt}$	$\mu\text{g/g dry wt}$
Blue Rose	5	0.76	0.114
Early Prolific	5	0.56	0.126
Nira	3	0.53	0.124
Rexoro	5	0.54	0.133
American Pearl	2	0.49	0.117
Fortuna	7	0.52	0.123
Composite average		0.57	0.121

The biotin values in Table I corroborated the trends exhibited by the other vitamin values, the vitamin content decreasing with increasing milling. Rice polish and bran were high in biotin content when compared with other foods (Lampen, Bahler, and Peterson, 1942).

The results reported in Table II do not show appreciable differences among the different varieties. The trend for the long-grained varieties, such as Fortuna and Nira, to lead in vitamin content was not demonstrated so clearly as in the earlier half of the present study.

### Summary

Samples of different milled fractions of several varieties of rice were analyzed for riboflavin and biotin. Values obtained for riboflavin agreed closely with those of other workers; biotin values were somewhat higher than the few figures for isolated samples reported by other workers, but appeared to be in agreement with assays obtained at another laboratory.

All samples of milled fractions showed that the biotin and riboflavin content of the rice samples decreased with increased milling, as has been demonstrated for thiamine, nicotinic acid, pantothenic acid, and pyridoxine in the first part of the present study. In general, 66 to 75% of all six of the vitamins studied is removed in the milling process, and polished rice contains only about 12% of the vitamin content of rice polish.

No appreciable differences in riboflavin and biotin content were found among the several varieties.

### Acknowledgment

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## SIMPLIFICATION OF THE THIOCHROME METHOD FOR THIAMINE DETERMINATION

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(Received for publication July 8, 1944)

With the widespread enrichment of flour there has come a need for a rapid yet reliable technique for the determination of thiamine in mill products and in bread. The thiochrome procedure given in *Cereal Laboratory Methods* (4th ed., 1941) appears to have been satisfactory in most respects but it is too time-consuming to be entirely useful as a routine control method. Several suggestions have previously been made as to means of simplifying the original thiochrome technique. Andrews and Nordgren (1941) found that the method could be much shortened by omitting the purification step of adsorption on zeolite. In their simplified method the sample is merely shaken with a 25% solution of potassium chloride, in dilute acetic acid, filtered, and an aliquot then oxidized in the usual manner. This technique, while apparently satisfactory for patent and first clear flours, has not been recommended for use with other products. Their studies would indicate, however, that various cereal products may be analyzed without the use of zeolite adsorption. Hoffer, Alcock, and Geddes (1943) studied a modification of the simplified procedure of Andrews and Nordgren and found that it was necessary to apply correction factors depending upon the product being analyzed.

The present paper describes a reasonably rapid method which can be applied to a wide variety of cereal products. As with other short methods, the zeolite adsorption has been eliminated in our modification. A single sample can be analyzed in 1 hr and one operator can analyze nine samples in 2½ hr with ease. The procedure is exactly the same for all mill products, and no correction factors need be used. In the case of bread analyses, the time required is somewhat longer; in our experi-

<sup>1</sup> Author's present address is Arnold Milling Company, Sterling, Kansas.

ence a digestion period of 2 hr is required to complete the breakdown of cocarboxylase, which extends the time required for analyses by 90 min.

### Proposed Simplified Thiochrome Method

#### Apparatus

- (1) Fluorophotometer
- (2) Centrifuge and 50-ml centrifuge tubes
- (3) Erlenmeyer flasks, 250 ml
- (4) Boiling water bath
- (5) Cold-finger condensers
- (6) Incubator, set at 50° C
- (7) Test tubes, 50-ml
- (8) Glass-stoppered shaking bottles, 25-ml

#### Reagents

- (1) Acetic acid solution, 2%
- (2) Sodium hydroxide, 1*N*
- (3) Takadiastase solution, 6%, made up fresh daily
- (4) Potassium ferricyanide solution, 1%. If kept in a dark bottle away from light this solution will last a week or more.
- (5) Quinine sulfate solution. Dissolve sufficient quinine sulfate in 0.1*N* sulfuric acid to give a solution containing 1  $\mu$ g quinine sulfate per ml
- (6) Sodium hydroxide, 15%
- (7) Isobutanol. This reagent should not give a blank reading above 4 units. Used isobutanol can be reclaimed by treatment with activated carbon (Pader, 1943)
- (8) Standard thiamine solution. Make up in 2% acetic acid, a stock solution containing 10  $\mu$ g thiamine per 25 ml. Store in a cold place away from light
- (9) Potassium chloride, crystals, C.P.
- (10) Sodium sulfate, anhydrous, technical grade

#### Procedure

*Preparation of Extract.* The weight of sample taken for extraction should be varied according to the expected thiamine content. The quantities shown below are recommended.

Expected thiamine content	Sample weight
<i>mg/lb</i>	<i>g</i>
0 -1.0	4.0
1.1-2.5	2.0
2.6-5.0	1.0
Over 5.0	0.5

Place the selected quantity of the material to be tested in a 250-ml Erlenmeyer flask, add 40 ml of 2% acetic acid, shake, insert cold-finger condenser, and place in boiling water bath for 15 min. Remove flask and place in cold water until the temperature of the solution drops to approximately 50°C. Add 5 ml of 1*N* NaOH, shaking solution during addition. Add 5 ml of 6% takadiastase solution, stopper flasks, and place them in an incubator at 50°C. After 30 min filter through Whatman No. 1 or equivalent paper. Pipette a 5-ml aliquot of the solution into a shaking bottle. The extract is now ready for oxidation.



Subject to the same treatment a control solution containing 1  $\mu\text{g}$  of thiamine per 5 ml of solution. To 25 ml of the thiamine stock solution, add 15 ml of 2% acetic acid, 5 ml of NaOH, and then 5 ml of takadiastase solution. Place in incubator for 30 min, filter, and pipette a 5-ml aliquot into a shaking bottle.

*Oxidation.* It is usually convenient to oxidize two samples at a time. To each sample add approximately 0.5 g of KCl crystals and shake vigorously for a few seconds. Add 0.1 ml (2 drops) of 1%  $\text{K}_3\text{Fe}(\text{CN})_6$  solution and shake again. Add 3 ml of 15% NaOH solution and shake a third time. Add 15 ml of isobutanol, stopper, and shake vigorously for 1 min. Pour into a 50-ml centrifuge tube and centrifuge until any emulsion is broken. Separate the two layers and pour the alcohol phase into a test tube. Add enough anhydrous sodium sulfate to remove any remaining water and shake until a clear solution is obtained. Pour into a clean dry cuvette and measure fluorescence in the fluorophotometer which has previously been set with the quinine sulfate standard. The reading obtained is proportional to the amount of thiamine present in the sample plus any nonthiamine fluorescent impurities.

*Blank Determination.* Carry out a blank determination for each different type of product analyzed and for the control solution. To 5 ml of sample extract or of control solution add, in this order, KCl crystals, isobutanol, and NaOH. Stopper the bottle and shake for 1 min. Separate alcohol phase, dry, and measure the fluorescence as described above.

*Calculation.* Determine the amount of thiamine present from the following relation:

$$\text{Thiamine content, mg/lb} = \frac{R_x - R_{xB1}}{R_y - R_{yB1}} \times \frac{50}{5} \times \frac{1}{Z} \times 0.454, \text{ where}$$

$R_x$  = Reading for unknown

$R_y$  = Reading for Standard Thiamine Control Solution

$R_{xB1}$  = Reading for blank carried out with unknown

$R_{yB1}$  = Reading for blank carried out with thiamine control solution and

$Z$  = grams sample taken

### Accuracy of Simplified Method

In Tables I and II are given a summary of the results of the collaborative analysis of 127 samples of a variety of cereal products by five laboratories. The samples are grouped according to their approximate thiamine content because, as shown by Hildebrand and Geddes (1943), the experimental error of the thiochrome method increases with increasing thiamine level. Four of the five laboratories employed the

TABLE I  
MEAN THIAMINE RESULTS OBTAINED BY REGULAR AND  
MODIFIED THIOCHROME PROCEDURES

Sample		Thiamine range	Regular method					Simplified method E
Type	Number analyzed		A	B	C	D	Avg.	
		mg/lb	mg/lb	mg/lb	mg/lb	mg/lb	mg/lb	mg/lb
Short patent	20	0.15-0.35	0.28	0.28	0.28	0.27	0.28	0.28
Long pat.; st. grade	21	0.50-0.80	0.57	0.57	0.58	0.58	0.58	0.60
Durum; white rye	6	0.75-1.50	1.06	1.03	1.09	1.01	1.05	1.04
First clear	17	1.30-1.60	1.45	1.43	1.48	1.50	1.46	1.49
Enriched pat.	20	1.45-2.25	1.78	1.72	1.75	1.82	1.77	1.84
Whole wheat	23	1.85-2.35	2.15	2.15	2.13	2.06	2.12	2.15
Noodles; dk. rye; oat flr.	10	2.50-5.50	3.39	3.41	3.40	3.38	3.40	3.37
Second cl.; red dog; germ	10	7.00-10.50	8.64	8.47	8.14	8.24	8.37	8.37
All samples	127	0.15-10.50	2.05	1.97	1.96	1.96	1.98	1.99

TABLE II  
ERRORS OF REGULAR AND MODIFIED THIOCHROME PROCEDURES<sup>1</sup>

Sample		Regular method					Simplified method E
Type	Number analyzed	A	B	C	D	Avg.	
		mg/lb	mg/lb	mg/lb	mg/lb	mg/lb	mg/lb
Short patent	20	0.042	0.043	0.031	0.020	0.034	0.021
Long pat.; st. grade	21	0.069	0.067	0.033	0.022	0.048	0.036
Durum; white rye	6	0.041	0.049	0.072	0.044	0.052	0.040
First clear	17	0.294	0.236	0.295	0.157	0.246	0.180
Enriched pat.	20	0.173	0.357	0.257	0.213	0.250	0.269
Whole wheat	23	0.376	0.354	0.308	0.315	0.338	0.274
Noodles; dk. rye; oat flour	10	0.984	0.486	0.964	0.737	0.793	0.856
Second cl.; red dog; germ	10	0.417	0.496	0.502	0.405	0.455	0.459
All groups	127	0.265	0.249	0.265	0.210	0.248	0.231

<sup>1</sup> Errors were calculated from the relation

$$E = \sqrt{(x_1 - m_1)^2 + (x_2 - m_2)^2 + \dots}$$

$$N - 1$$

where

$x_1, x_2$ , etc. = individual laboratory values for samples 1, 2, etc.

$m_1, m_2$ , etc. = mean values, all laboratories, for samples 1, 2, etc.

and

$N$  = number of samples.

thiochrome method essentially as described in Cereal Laboratory Methods (4th ed., 1941), while the values listed under "Simplified Method" are characteristic of the procedure described in this paper. It is evident that the accuracy of the modified method is entirely satisfactory.

### Summary

A simplification of the thiochrome method for thiamine analysis is described. The short method effects a significant saving in time and effort, can be applied to all types of flour mill products and to many

other types of cereal products, and requires no correction factors. No changes need be made in the method except for the analysis of baked goods.

The accuracy of the simplified method, as shown by the collaborative analysis of 127 samples of various cereal products, compares favorably with that of the regular thiochrome method.

The simplified method is admirably suited to routine control determinations.

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## THE COMPOSITION OF THE "AMYLODEXTRIN" FRACTION OF WHEAT FLOUR

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The so-called "amylodextrin" or "small-granule" fraction of wheat flour is of importance both in enhancing the baking properties of flour and in the commercial production of wheat starch. Sandstedt, Jolitz, and Blish (1939) have reported that this fraction contributes to the handling characteristics of dough and is essential for the production of bread with a tender crumb. In the production of wheat starch, the "amylodextrin" or "tailings" fraction has significance because it is an almost profitless by-product. In commercial operations, after removal of the starchy material from the gluten mass by washing with water, the starch is separated by tabling. The flocculent material of low density, that is "amylodextrin" fraction or tailings starch, which flows over the end of the table is recovered and converted into a low-grade and low-priced adhesive. Since this fraction may represent as much as 20% of the starch, information concerning its composition would be of use in the development of a more profitable outlet.

<sup>1</sup> This is one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Considerable confusion has arisen in the literature owing to the conflicting statements concerning the actual composition of the "amylo-dextrin" fraction of wheat flour. Sandstedt, Jolitz, and Blish (1939) considered it to be composed of dextrans produced by amylase activity. The presence of large numbers of small starch granules was reported by Goldbeck (1916) and by others. Pentosans represent 14% by weight of the small-granule fraction according to Baker, Parker, and Mize (1943), who regarded them as being adsorbed upon the relatively large surface area of the small granules. It was believed by these authors that the coating of pentosan on the starch granules might account for many of the properties of the fraction.

In the present investigation, microscopic and analytical methods have been used to determine the composition of the "amylo-dextrin" fraction.

### Experimental

*Preparation of Fractions.* Two 1941 crop year wheats were used for this study (which was carried out chiefly in 1942): (1) Turkey hard red winter, grown at Manhattan, Kansas, graded No. 1 dark hard, and (2) Trumbull soft red winter, grown at Wooster, Ohio, graded No. 1 red winter. Each contained only a trace of dockage which was removed before the wheat was weighed for use.

Starch and "amylo-dextrin" fractions were separated from three samples of each wheat: (1) whole wheat kernels, steeped 24 hr in distilled water at approximately 5°C, and subsequently ground twice through a Russwin No. 2 handmill, using the nut-butter cutter, (2) straight flour obtained by milling the wheat on a laboratory-model Buhler mill, and (3) flour obtained by remilling the original flour twice through the Buhler mill.

Each sample was extracted as follows: 400 g of ground sample (original air-dried weight) was worked into a stiff dough with distilled water and allowed to stand for 1 hr at room temperature to facilitate formation of a "gluten ball." Starch was extracted from the hydrated mass by working it in successive portions of distilled water. Fine fiber was removed by passing the combined extraction liquors, totaling 6 to 8 l in volume, through No. 13 standard bolting silk. The starch and "amylo-dextrin" fractions were centrifuged from the liquid and washed once with distilled water after which the upper, "amylo-dextrin" layer was carefully removed from the starch with a spatula. The starch was resuspended in distilled water and recentrifuged, the residual traces of the "amylo-dextrin" fraction being removed as before. The remaining starch was washed twice with distilled water and air-dried at room temperature. Since the "amylo-dextrin" layer became horny when dried from water, it was, except during drying studies and for analysis,

air-dried at room temperature after removal of water by three or more acetone washes.

*Analytical Methods.* The composition of the starch and "amylo-dextrin" fractions was determined by standard analytical procedures. The moisture in the "amylo-dextrin" and starch fractions was determined by drying to constant weight at 100°C over  $P_2O_5$  in an Abderhalden drier; in wheat, by drying the ground grain for 1½ hr in a Brabender Moisture Tester at 130°C. Determinations were made for nitrogen by the Kjeldahl-Gunning-Arnold method (A.O.A.C., 1940, p. 26), starch by a diastase method (A.O.A.C., 1940, p. 359), and fatty acids or fats by acid hydrolysis and extraction from the hydrolysate. Pentosans were determined as furfural by a modified method developed by the Analytical and Physical Chemical Division of the Northern Regional Research Laboratory. The distillate obtained by the official A.O.A.C. (1940) procedure was redistilled and the furfural was precipitated by thiobarbituric acid. In this way erroneously high results due to measurement of the hydroxymethylfurfural formed from hexose material were avoided.

*Examination of Starch and "Amylodextrin" Fractions.* Starch and "amylo-dextrin" fractions prepared from whole wheat by wet milling, and from once- and thrice-milled flour were examined microscopically, analytically, and from the standpoint of yields.

More starch was recovered from wheat flour than from whole wheat because of the larger percentage of starch present in the former. The yield of starch fraction decreased upon remilling the flour from soft wheat, but was not significantly affected by the remilling of hard wheat flour (Table I). Photomicrographs of starch fractions from Turkey wheat are shown in Figure 1; the fractions from Trumbull wheat had a similar microscopic appearance. Starch fractions obtained from the various raw materials showed no unusual characteristics or differences. In each case, the nitrogen content of the starch was normal, and pentosans were absent. More small granules were freed by milling through the Buhler mill than by use of the handmill (Figs. 1A and 1B). No appreciable granule damage was observed (Figs. 1A, 1B, and 1C); birefringence was clear (Fig. 1D).

"Amylodextrin" fractions, on the other hand, varied considerably with the milling method, the yield increasing significantly with increasing severity of grinding. (See Table I.) Photomicrographs of these fractions are shown in Figure 2. These fractions were found to be composed primarily of very small starch granules, their average size being comparable to that of the smallest granules in the starch fraction (Figs. 2A, 2B, and 2C). In addition, large granules were present in varying amounts. From their partial or complete loss of birefringence

(Fig. 2D) and their ready absorption of stains, such as benzopurpurin or congoecorinth, it was apparent that practically all the larger granules had suffered damage and, in consequence, were partly, often completely, gelatinized in the liquid in which they were mounted for observation. The number of damaged granules increased with severity

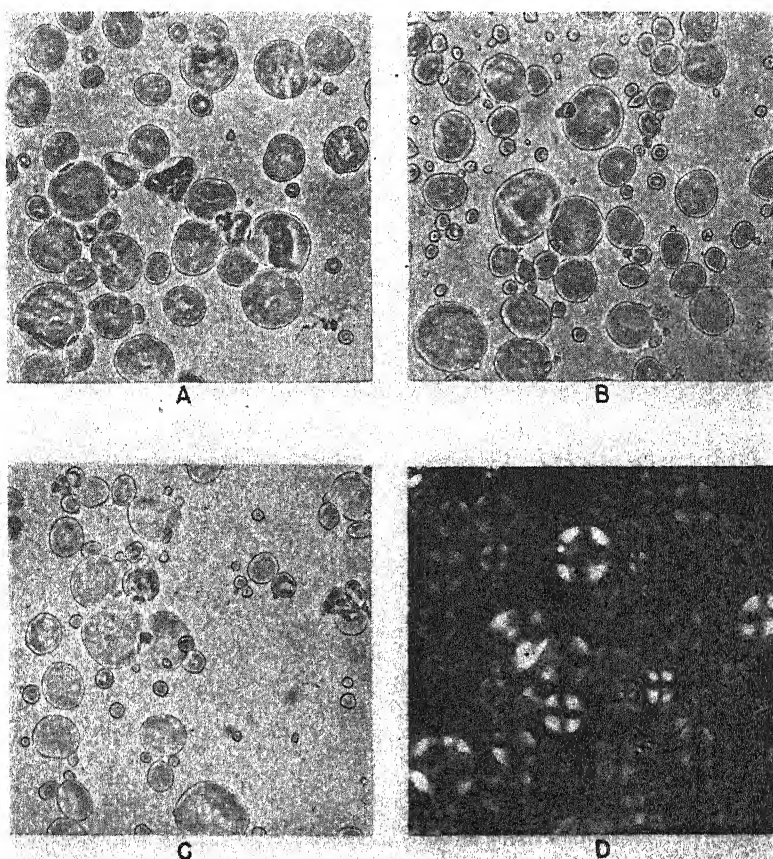


Fig. 1. Starch from Turkey wheat. (Magnification 285 X)

- A. Extracted after wet milling.
- B. Extracted from flour.
- C. Extracted from twice remilled flour.
- D. Same field as B, between crossed nicols.

of grinding, being least when the handmill was used and most in the twice-remilled material (Figs. 2A and 2C). All the "amyloextrin" fractions included small gluten masses and relatively large fragments, from the microscopic standpoint, of cell debris.

The analytical data confirmed the microscopic evidence that the

"amyloextrin" fraction was composed largely of starch, which, in all cases, comprised 87 to 94% of the fraction. Nitrogen analyses showed the presence of from 0.9 to 1.8% protein ( $N \times 5.7$ ). Protein increased with the extent of milling in the case of hard wheat, but fluctuated less in the case of soft wheat.

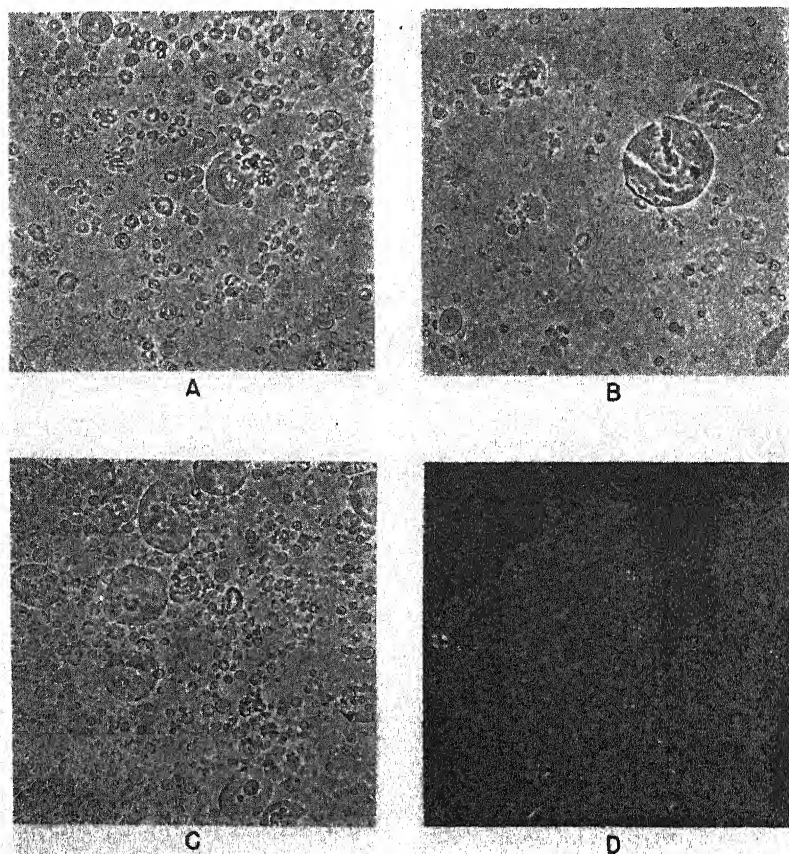


Fig. 2. "Amyloextrin" fraction from Turkey wheat. (Magnification 285 X)

- A. Extracted after wet milling.
- B. Extracted from flour.
- C. Extracted from twice remilled flour.
- D. Same field as C, between crossed nicols.

A more complete analysis of a typical "amyloextrin" fraction is shown in Table II. The major nonstarch materials were pentosans, protein, fatty acid, and ash. Less than 3.5% of the fraction was unidentified and this is believed to be essentially cellulosic material in the cell wall fragments. A cellulose determination of so small an

TABLE I  
YIELD OF AND ANALYTICAL DATA ON STARCH AND "AMYLODEXTRIN" FRACTIONS  
All data on dry basis

Sample	"Amylodextrin" fraction			Starch fraction	
	Yield	Nitrogen	Starch	Yield	Nitrogen
	%	%	%	%	%
Turkey, whole wheat	9	0.16	94.6	50	0.04
Turkey, flour	10	0.25	91.1	60	0.05
Turkey, twice remilled flour	15	0.32	89.6	59	0.05
Trumbull, whole wheat	8	0.32	87.5	49	0.04
Trumbull, flour	12	0.24	90.1	67	0.05
Trumbull, twice remilled flour	15	0.29	89.0	60	0.05

TABLE II  
COMPOSITION OF "AMYLODEXTRIN" FRACTION FROM TURKEY  
WHEAT FLOUR (ON DRY BASIS)

Starch	Pentosans	Protein ( $N \times 5.7$ )	Fatty acids	Ash
%	%	%	%	%
90.1	4.0	1.5	0.7	0.27

amount would not have been practical, however, and was not attempted. Rough microscopic estimation suggested that the amount of cell debris present was approximately of the same order as the pentosans found by analysis. That the pentosans were largely in the cell debris was further supported by the fact that the starch fraction, which contained no cell wall material, also contained no pentosans. The fatty acid content of the fraction was about the same as that of prime-quality starch (0.7%), and, presumably, the fat was present mainly as a component of the starch granules. The ash, however, was twice as great as that of ordinary wheat starch.

The most noticeable characteristic of the small-granule fraction is its semifluid nature. Density determinations made by pycnometer measurements gave:

"Amylodextrin" fraction	1.453
Methanol-extracted "amylodextrin" fraction	1.463
Starch fraction	1.485

The effect of treating the small-granule fraction with amylase was investigated in order to determine if this treatment facilitated the preparation of the small-granule fraction in a pulverulent form, the development of such a procedure being of value in the commercial processing of flour for starch. Removal of the gelatinized damaged granules by enzyme treatment only slightly lessened the semiliquid



nature of the fraction. On drying the treated fraction in air, a horny intractable product was obtained. Apparently the pentosan and protein components, rather than gelatinized starch granules of the fraction, are mainly responsible for the great difficulty in obtaining the fraction as a dry, friable powder by ordinary dehydration.

### Discussion

In this work it has been shown that the small-granule or "amylo-dextrin" fraction comprises starch (87–94%), protein (1–2%), pentosans (4%), fatty material (about 0.7%), and ash (about 0.3%). In addition there is a small amount of cellulosic material, probably about 3%. The starchy material is composed of ungelatinized small granules and damaged, large granules which gelatinize partially or wholly in cold water, the former predominating. The observations on the presence of small granules confirm the work of Goldbeck (1916) and others. The amounts of protein and fatty material found in the small-granule fraction are approximately in agreement with values previously reported by Sandstedt, Jolitz, and Blish (1939). Data on pentosan content, however, are of a different order than those given by Baker *et al* (1943), who reported 14% as compared to about 4% found in this work. It is difficult to explain this discrepancy. Baker *et al* do not give the starch content of their "amylo-dextrin" fraction which was extracted with a salt solution. Since the complete analysis is not available, it is impossible to judge whether their "amylo-dextrin" fraction was dissimilar to those studied by the present writers.

An outstanding characteristic of the small-granule fraction is its semifluid nature, which cannot be attributed to density alone. The large difference in volumes (per unit weight of dry substance) of the starch and "amylo-dextrin" layers after centrifuging indicates a difference in the packing of the particles. After centrifuging, a layer of ordinary starch is somewhat plastic (essentially solid) indicating close packing of the granules, whereas the layer of small-granule starch is viscous (essentially fluid) indicating extremely loose packing. Other starches consisting of small granules tend to have similar characteristics. This may be due to the much larger ionic charge per unit volume that small granules possess, the effect of a charge on a particle becoming more pronounced as the size of the particle decreases and its surface area per unit volume increases. The high ash content of the small-granule fraction suggests that a considerable part of the inorganic matter may be adsorbed by the particles, thus increasing their polarity. Particle size is not, however, the sole factor influencing consistency. Rice starch, with granules approximately equal in size to the small granules of the "amylo-dextrin" fraction, is less fluid than the latter,

but more fluid than prime-quality corn or wheat starch. Further work would be necessary to determine whether small starch granules of different species exhibit variations in packing owing to differences in shape, etc. The low density and large area of the pieces of cell debris probably also contribute materially to the physical nature of the fraction as a whole.

It would appear desirable to avoid designation of the small-granule fraction by the name "amylodextrin." Amylodextrin is a recognized enzymic degradation product of starch. Unlike the small-granule fraction, it is water soluble. The name "small-granule fraction" has a disadvantage in that important properties of the material appear to be due to components other than the small granules. "Tailings fraction" might be a more acceptable term, since the fraction separated by centrifuging is essentially the same as that flowing over the tail when crude starch is tabled, and since the name has no implications as to chemical composition.

### Summary

The so-called "amylodextrin," small-granule or tailings fraction separated by centrifuging crude wheat starch after the removal of the bran and gluten, is composed of very small starch granules, varying amounts of damaged large granules (depending upon severity of milling), and smaller quantities of protein, fatty acids, ash, and pentosans, and cellulose in the cell wall debris. The damaged granules gelatinize in cold water. There is evidence of relatively little degradation of the starch.

Large sorptive surfaces of the small granules, together with high ash content, suggest that the semifluid consistency of the fraction is due at least in part to an electroviscous effect.

### Acknowledgments

Wheat samples were collected and dry milled by the Commodity Development Division, and starch, nitrogen, ash, pentosan, and fatty acid determinations were made by the Analytical and Physical Chemical Division, of this Laboratory. The assistance of C. W. Bice, J. W. Eck, and H. H. Rhodes in the laboratory experiments is gratefully acknowledged.

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# REPORT OF 1943-44 COMMITTEE ON RELATION OF LABORATORY BAKING TO SHOP PRACTICE

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(Read at the Annual Meeting, May 1944)

Six samples of commercially acceptable bread flour, as listed in Table I, were submitted to seven collaborators with a request to bake and evaluate them in accordance with their existing methods and report the following information for each sample: (1) mixing time and type of mixer, (2) fermentation time and yeast percentage, (3) fermentation temperature, (4) absorption (13.5% moisture basis), (5) proof time and temperature, (6) baking time and temperature, (7) loaf volume, (8) scaling weight of loaf, (9) weight of loaf from oven. Identical data on the same samples were obtained from commercial shops.

TABLE I  
FLOURS EMPLOYED IN COLLABORATIVE STUDY

Sample No.	Flour type	Protein <sup>1</sup>	Ash <sup>1</sup>
		%	%
1	Spring	12.2	0.40
2	Spring	12.3	0.42
3	Winter	11.9	0.39
4	Winter	12.4	0.44
5	Spring	12.5	0.46
6	Winter	12.4	0.47

<sup>1</sup> 13.5% moisture basis.

The baking methods used by the various collaborators covered a rather wide range of testing procedures. Two laboratories used both sponge and straight dough methods of baking, one used the sponge procedure exclusively, while the remainder used straight dough methods exclusively. Three operators used the "one hundred-gram" loaf test, while the remainder baked one-pound loaves. Six collaborators used variable absorption with one employing a fixed absorption; four employed varying mixing times with three using a constant mixing time for all flours. Six of the laboratories were equipped with Hobart-Swanson type mixers or the McDuffy bowl in connection with a Hobart mixer; the remaining collaborator employed a Washburn Crosby type mixer. Four varied fermentation and proofing times, while three held these constant. All collaborators used the same fermentation and proofing temperatures.

The mixing times are recorded in Table II. Collaborators 4, 5, 6, and 7 all employed a mechanical means of testing the flours for

TABLE II  
MIXING TIMES

	Sample No.					
	1	2	3	4	5	6
Col. 1	8	8	8	8	8	8
Col. 2	3	3	3	3	3	3
Col. 3	3	3	3	3	3	3
Col. 4	4	3½	2½	2½	4	3
Col. 6	3	3	2	2½	3¼	2½
Col. 7	3½	3⅝	2¼	2½	4	2⅞
4 Sponge	3½	3	2	2	3½	2½
5 Sponge	6	6	4	4	6	6
6 Sponge	3½	3	2¼	2¼	3½	3
Commercial	8½	8½	9	9	8½	9

mixing time. Collaborator 5 used a fixed time only in the dough stage of his sponge and dough procedure, and the time listed for this operator is that at which his best results were obtained. Where mixing time was varied, there was rather close agreement between operators. Increased mixing time for the commercial shop on samples 3 and 4 is due to the softer dough as noted in Table III. A definite tendency toward longer mixing time for the spring wheats is also evident.

TABLE III  
ABSORPTIONS (13.5% moisture basis)

	Sample No.					
	1	2	3	4	5	6
Col. 1	64	64	64.5	64.5	64.5	
Col. 2	59	59	59	59	59	59
Col. 3	62.3	62.5	64.9	65.2	61.8	64
Col. 4	63	62.5	65	66	62.5	63
Col. 6	64.2	64.8	64.8	64.6	64.8	64.8
Col. 7	63.5	61	61.5	62	64	63
4 Sponge	64.5	64.0	66.5	67.5	64	64.5
5 Sponge	62	62	64	63	63	63
6 Sponge	64.5	65	65	64.8	65	65
Commercial	63.5	63.2	62	65	63.5	63.2
Average	63.0	62.7	63.9	64.0	63.1	63.2

The absorption data are given in Table III. Collaborator 3 employed a farinograph to determine absorption, while the others relied on feel and experience, and it is quite evident that the personal equation has markedly influenced the results. The average absorption values of all collaborators follow the same trend as the commercial figures. The winter wheats were given slightly higher absorption than spring wheats.

TABLE IV  
FERMENTATION TIME IN MINUTES

	Sample No.					
	1	2	3	4	5	6
Col. 1	240	240	270	240	240	
Col. 2	180	180	180	180	180	180
Col. 3	180	180	180	180	180	180
Col. 4	113	111	106	108	111	110
Col. 6	180	172	142	142	180	146
Col. 7	180	180	180	180	180	180
4 Sponge	260	260	260	260	260	260
5 Sponge	340	340	340	370	370	340
6 Sponge	270	270	250	250	270	250
Commercial	270	260	225	215	240	360

The various fermentation times employed are recorded in Table IV. Three collaborators did not vary their fermentation, while the remainder showed some range of fermentation. Because of the wide range in the quantity of yeast employed, a second table has been prepared showing the fermentation times in minutes per percent of yeast (Table V).

TABLE V  
FERMENTATION TIME IN MINUTES PER PERCENT YEAST

	Sample No.					
	1	2	3	4	5	6
Col. 1	101	101	114	101	101	
Col. 2	72	72	72	72	72	72
Col. 3	60	60	60	60	60	60
Col. 4	56.5	55.5	53	54	55.5	55
Col. 6	75	71	59	59	75	60
Col. 7	90	90	90	90	90	90
4 Sponge	87	87	87	87	87	87
5 Sponge	122	122	122	128	128	122
6 Sponge	78	78	69	69	78	69
Commercial	78	75	73	66	78	X

Employing the sponge method, collaborator 6 adopted a fermentation time which agrees rather closely with that used by the commercial shop. The others who used variable fermentation times all gave the spring wheat flours more fermentation than the winter wheat flours. This is true also in the commercial tests. These results indicate that the fermentation times employed by an individual laboratory might correlate satisfactorily with those of a given commercial bakery.

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TABLE VI  
PROOF TIME

	Sample No.					
	1	2	3	4	5	6
Col. 1	58	58	57	58	58	
Col. 2	40	40	40	40	40	40
Col. 3	55	55	55	55	55	55
Col. 4	54	54	55	53	52	48
Col. 6	65	65	64	63	62	60
Col. 7	55	55	55	55	55	55
4 Sponge	49	50	47	48	45	42
5 Sponge	70	72	70	69	65	58
6 Sponge	67	68	70	68	65	58
Commercial	60	59	55	58	60	

The proof times recorded in Table VI show less variation between operators than the data for mixing and fermentation times. Where proof time was varied there was a trend toward shorter proofing time with the samples having the highest protein.

TABLE VII  
LOAF VOLUME IN CC/G OF DOUGH

	Sample No.					
	1	2	3	4	5	6
Col. 1	5.16	5.13	5.12	4.97	5.02	
Col. 2	4.15	4.05	3.94	4.19	4.45	4.28
Col. 4	4.25	4.27	4.20	4.25	4.25	4.22
Col. 6	5.80	5.90	5.6	5.50	5.78	5.65
Col. 7	4.94	4.91	4.79	4.89	5.31	4.86
4 Sponge	4.39	4.40	4.39	4.52	4.52	4.35
5 Sponge	4.89	4.92	4.72	4.79	4.75	4.75
6 Sponge	5.90	6.0	5.7	5.62	5.85	5.72
Avg. <sup>1</sup>	4.86	4.85	4.73	4.76	4.96	4.75
Avg. <sup>2</sup>	5.06	5.10	4.89	4.97	5.04	4.94
Commercial	4.59	4.55	4.48	4.60	4.68	4.53

<sup>1</sup> Average straight dough.

<sup>2</sup> Average sponge dough.

The loaf volumes expressed on a unit weight of dough are presented in Table VII. The average loaf volumes for all collaborators rank the flours in the same order as their protein content.

The results of this study serve to emphasize some of the difficulties involved in attempting to correlate laboratory baking results with shop practice. The problem of correlating the absorption and mixing used in the laboratory with shop practice is relatively simple, since the instruments such as the micromixer and farinograph are available

for these purposes. It would seem necessary to employ a fixed formula, preferably that of the baker, and vary the fermentation to obtain either an optimum loaf or one that corresponds to that produced under shop conditions. Proof time could either be held constant or varied to give a loaf having a similar specific volume to that produced commercially. Studies of the relation between experimental and commercial baking results should be continued. The ultimate goal can best be achieved by complete cooperation between the cereal chemist and the practical baker. It is therefore suggested that consideration be given to the possibility of further studies being undertaken as a cooperative effort of the cereal chemists and the bakery engineers.

#### Acknowledgments

The members who collaborated in this work were: Ray Bohn, Karl Finney, E. N. Frank, J. Freilich, J. H. Lanning, R. W. Mitchell, Ray Powers, and D. B. Pratt.

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## THE RELATIONS BETWEEN KJELDAHL PROTEIN, ZELENY PROTEIN, AND LOAF VOLUME IN WESTERN CANADIAN WHEATS AND FLOURS<sup>1</sup>

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(Presented at the Annual Meeting, May 1944; received for publication May 26, 1944)

About four years ago, Zeleny (1941) introduced a new simple procedure for determining protein content in wheat and flour. The proteins are peptized in a dilute alkaline solution, a stable colloidal suspension of the proteins is produced by a controlled change of the hydrogen-ion concentration, and the resulting turbidity is measured in a photometer by transmitted light. Zeleny found a close association for flours between light transmission and protein not peptized by 5% potassium sulfate solution, and Zeleny, Neustadt, and Dixon (1942) also found a close association for wheats between light transmission and a calculated value for endosperm protein. For these reasons they stated that "... the results obtained are probably a somewhat better index of baking quality than are the values obtained by the conventional Kjeldahl procedure."

This hypothesis and the simplicity of the method suggested that further investigation might prove profitable. Accordingly, a study was made of the relations between loaf volume, Zeleny protein, and

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Kjeldahl protein. Baking strength, as measured by loaf volume, is only one of the components of bread-making quality in flour; but it is an important one, which also lends itself to quantitative study. Although no closer relation was found between Zeleny protein and loaf volume than between Kjeldahl protein and loaf volume, the results of the study are of sufficient interest to warrant publication.

### Materials and Methods

When assessing the ability of any method for estimating loaf volume, it is advisable to investigate both inter- and intravarietal relations. For this purpose, the ideal series of samples comprises a number of different varieties, each grown at a number of different stations. Unfortunately it was not possible to obtain such a series, and a compromise had to be made by using one series of samples to study intervarietal relations and another to study intravarietal relations.

The first series consisted of composites of 25 varieties that had been grown in the "Uniform Variety Tests" by the Dominion Department of Agriculture at nine different stations in Western Canada during 1943. The second series consisted of 50 samples of Thatcher wheat grown by registered seed-growers at 50 different points throughout Western Canada in the same year. All samples graded 3 Northern or higher.

The wheats, which were cleaned free from dockage, were milled in an Allis-Chalmers experimental mill, and a standard feed flour was used to control the yields. Flours of approximately 71% extraction were obtained and these were baked in triplicate by the standard malt-phosphate-bromate formula.

Protein content was determined in duplicate on duplicate subsamples of the wheats, and in quadruplicate on the flours, by the Kjeldahl-Gunning-Arnold method. Corresponding replicate determinations of light transmissions were made by the procedure outlined by Zeleny *et al* (1942), with the use of an Evelyn colorimeter and a light filter of 540 m $\mu$ . However, instead of shaking the samples and the peptizing agent intermittently for 3 min, they were rotated 50 to 60 times per min for 5 min.<sup>2</sup> The wheats for the Kjeldahl method were ground through a burr mill with a close setting, and for the Zeleny method they were ground through a Wiley mill with a 1-mm sieve. The Kjeldahl proteins were corrected to a 13.5% moisture basis, and Zeleny transmission values were obtained on the same basis by appropriate adjustment of the amount of sample weighed out.

<sup>2</sup> This change was suggested in a private communication from Dr. L. Zeleny who pointed out that vigorous shaking appears to have some undesirable effect on the physical properties of the gluten and tends to cause somewhat erratic results.



## Results and Discussion

*Relations with Loaf Volume.* The primary object of the investigation was to determine whether or not the Zeleny method gives results that are more closely related to loaf volume than those given by the Kjeldahl method. This matter can be examined with the aid of the scatter diagrams in Figures 1 and 2. The former shows that the range

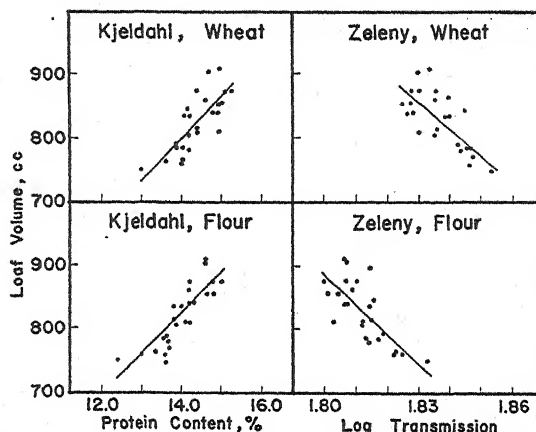


Fig. 1. Scatter diagram for the 25 varieties showing relations between Kjeldahl protein and loaf volume and Zeleny values and loaf volume.

of protein contents for the intervarietal series is quite narrow; but this is to be expected because the samples represent composites for varieties that are normal in protein content and that have been grown under the same environmental conditions. In the Thatcher series (Fig. 2) a comparatively wide range of protein contents is represented. It is hardly possible to tell by inspection of these figures whether the relations for Zeleny protein are closer than those for Kjeldahl protein, and the correlation coefficients must therefore be examined.

The simple correlation coefficients are shown in Table I. All are highly significant, but those for Zeleny protein and loaf volume are lower than those for Kjeldahl protein and loaf volume except in one instance in which they are equal. These differences, however, are not great enough to be statistically significant. Therefore, all that can be said is that the Zeleny method measures some fraction of the total protein that is no more closely related to loaf volume than is total protein determined by the Kjeldahl method.

As the Zeleny method measures some fraction of the total protein, and as this fraction is closely correlated with loaf volume, it is important to determine whether this relation is independent of bilateral

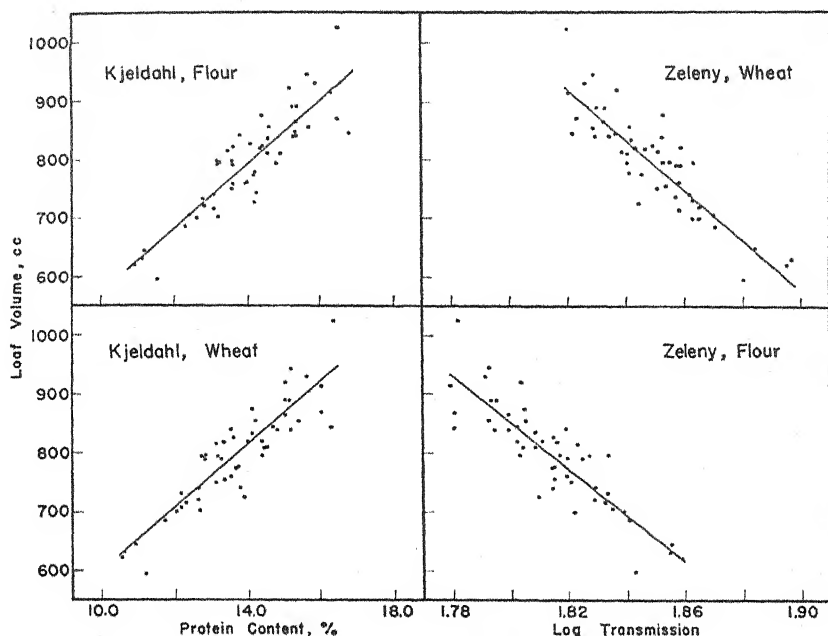


Fig. 2. Scatter diagram for Thatcher series showing relations between Kjeldahl protein and loaf volume and Zeleny values and loaf volume.

relations with total protein. Accordingly, the partial correlations of loaf volume with Zeleny protein independent of Kjeldahl protein were calculated. These partial correlations (Table I) are extremely small,

TABLE I

SIMPLE AND PARTIAL CORRELATION COEFFICIENTS FOR LOAF VOLUME WITH KJELDAHL PROTEIN AND WITH ZELENY PROTEIN

Series	Simple correlations		Partial correlations Loaf volume $\times$ Zeleny independent of Kjeldahl
	Loaf volume $\times$ Kjeldahl protein	Loaf volume $\times$ Zeleny protein	
25 Varieties			
Wheat	0.79 **	-0.75 **	-0.10
Flour	0.84 **	-0.78 **	-0.03
Thatcher			
Wheat	0.88 **	-0.88 **	-0.20
Flour	0.90 **	-0.88 **	0.22

Note: In this and subsequent tables, \*\* denotes 1% level of significance attained.

and thus tend to prove that the Zeleny method is measuring some moderately constant proportion of the total protein. Perhaps it should be emphasized here that these conclusions are based upon studies with wheats that contain few if any damaged kernels. Similar

tests with samples of light test weight containing damaged kernels might well lead to modified conclusions.

In this connection it is of interest to quote from a private communication from Dr. L. Zeleny: "The fact that globulins, albumins, proteoses, and other simpler nitrogenous compounds remain almost completely peptized in the medium used for the turbid suspension and the fact that the proteins of wheat bran have been shown to contribute but very little to the turbidity of the suspension make it appear reasonable to postulate that the photometric method is essentially, although probably not strictly, a measure of the gluten protein content of the wheat or flour. This hypothesis is supported by the further fact that although the Kjeldahl protein content of flour is invariably lower than that of the wheat from which the flour is milled, the photometric protein content of the flour is invariably higher than that of the wheat. Total protein ( $N \times 5.7$ ) is always lower in the flour than in the corresponding wheat, but gluten protein, on the other hand, is higher in the flour since most of the non-gluten proteins and but very little of

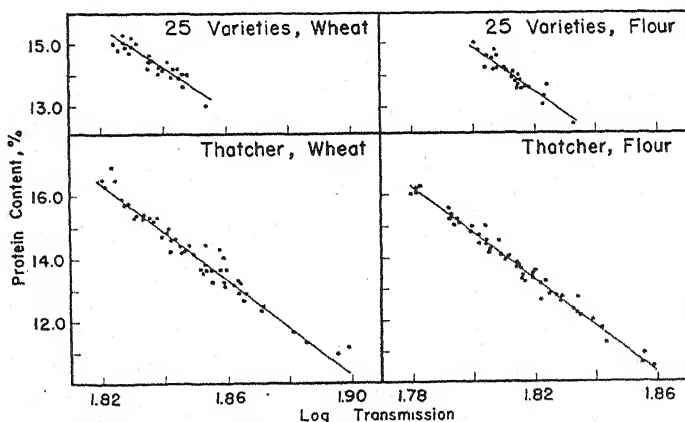


Fig. 3. Scatter diagram for the 25 variety and Thatcher series showing relation between Kjeldahl and Zeleny values.

the gluten protein are removed with the bran and shorts in the milling process." Figures 1 and 2 illustrate this last statement; for the Zeleny method flour proteins are much higher than those of the wheats from which they were milled (*i.e.* transmission values are lower for flours), whereas a slight difference, but in the opposite direction, is shown by the Kjeldahl data.

There are other comparisons of correlation coefficients that can be made from the data in Table I, but these are of secondary importance. The correlations for loaf volume and flour protein tend to be higher

than those for loaf volume and wheat protein, and the Thatcher correlations tend to be higher than the intervarietal correlations. These differences are in the expected directions, though they are not statistically significant.

*Relation between Kjeldahl and Zeleny Protein.* The data were also examined to determine whether the Zeleny method could be used to predict Kjeldahl protein. The scatter diagrams for the Zeleny-Kjeldahl relations are shown in Figure 3, and the corresponding correlation and regression coefficients are given below:

	Correlation coefficients		Regression coefficients	
	25 Varieties	Thatcher	25 Varieties	Thatcher
Wheat	-0.92 **	-0.98 **	-63	-76
Flour	-0.93 **	-0.99 **	-71	-74

All the correlation coefficients exceed the values required for the 1% level of significance, and indicate that Kjeldahl protein can be predicted from Zeleny protein with considerable precision. The standard errors of estimate from means of quadruplicate determinations are as follows:  $\pm 0.2\%$  protein for wheat and flour in the intervarietal series and for flour in the Thatcher series, and  $\pm 0.3\%$  protein for wheat in the Thatcher series. However, the same prediction equations cannot be used both within and between varieties. This follows from the fact that there are significant differences between the regression coefficients for the Thatcher and the intervarietal series. The statistics thus suggest that an accurate general equation for predicting Kjeldahl protein from Zeleny protein cannot be developed for a miscellaneous group of samples representing different varieties grown under various environmental conditions. Satisfactory prediction will probably be limited either to samples of one variety grown in different places, or to samples of different varieties grown in one place.

*Comments on the Zeleny Method.* The technique of the Zeleny method is quite simple, but the analytical error is appreciably higher than that of the Kjeldahl method. Unlike the latter, the Zeleny method gives higher errors for flours than for wheats. Flours are more difficult to test than wheats by the Zeleny method because it is not easy to paste them up thoroughly while the dispersing agent is being added. A comparison of the errors of the Kjeldahl and the Zeleny methods can be made from the following standard errors of duplicate determinations, which are recorded in terms of Kjeldahl protein:

	25 Varieties		Thatcher	
	Kjeldahl	Zeleny	Kjeldahl	Zeleny
Wheat	0.07	0.09	0.09	0.15
Flour	0.11	0.17	0.07	0.25

With the Zeleny method the greater part of the error appears to lie in the dispersing process, but it is reasonable to believe that further investigations may result in improvements that will materially lower the analytical error.

The cost of operating the Zeleny method is much lower than that of the Kjeldahl method, and the amount of laboratory space required is much smaller. The analytical operations of the Zeleny method can be carried out very rapidly, but more time is required for the preparation of wheat samples for this method than for the Kjeldahl procedure. The Zeleny method has special promise in field laboratories where it is not possible to install conventional protein apparatus.

### Summary

The photometric method developed by Zeleny for determining protein content in wheat and flour has been examined to determine whether it gives results that are more closely related to loaf volume than are conventional Kjeldahl data. The Zeleny correlations, both within one variety (50 samples of Thatcher: wheat,  $-0.88$ ; flour,  $-0.88$ ) or between 25 varieties (wheat,  $-0.75$ ; flour,  $-0.78$ ), were not as high as the corresponding Kjeldahl correlations ( $0.88, 0.90; 0.79, 0.84$ ).

The correlations between Zeleny values and Kjeldahl protein were  $-0.92$  and  $-0.93$  for wheat and flour in the intervarietal series, and  $-0.98$  and  $-0.99$  for wheat and flour in the Thatcher series. The Zeleny method can apparently be used for predicting Kjeldahl protein in sound samples of one wheat variety grown in different places, or in samples of different varieties grown at one place. It is improbable that a satisfactory general equation for all samples can be developed.

The evidence strongly suggests that the Zeleny procedure is measuring some relatively constant proportion of the total protein and that this protein fraction is concentrated largely in the endosperm.

The method is very simple, but, at the present time, the analytical error is higher than that of the Kjeldahl test.

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# IMPROVEMENTS IN THE DETERMINATION OF STARCH IN CORN AND WHEAT

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Methods for the determination of starch in materials of relatively high starch content, such as cereal foods, grains, and stock feeds, have been studied by the Association of Official Agricultural Chemists for the past several years. Present official methods ("Methods of Analysis, A.O.A.C.," 5th ed., 1940) for grain and stock feeds include (1) direct acid hydrolysis, (2) diastase method with subsequent acid hydrolysis, and (3) the latter with provision for the removal of interfering polysaccharides. The first of these methods is not applicable in the presence of pentosans known to occur in grains unless a large correction involving a separate analytical determination is made for pentosans. The second method and, especially, the third method are laborious and extremely time-consuming. These methods are usually avoided in routine analyses by industrial companies because of the time and labor involved. Under "Cereal Foods," the Rask method is listed by the Association of Official Agricultural Chemists as a tentative method, applicable to uncooked cereal products. This method is empirical, requiring very careful timing and strict adherence to every detailed direction. All of the above methods of the Association of Official Agricultural Chemists are repeated in the "Cereal Laboratory Methods, A.A.C.C." (4th ed., 1941). The unsatisfactory status of these methods is recognized by the continuing work of referees in the Association of Official Agricultural Chemists; and the most recent report (A.O.A.C., 1944) recommends the Hopkins (1934) revision of the Mannich-Lenz (1920) polarimetric procedure as a tentative method for cereal foods. Etheredge (1941) has surveyed starch methods, and Hopkins (1934) presents an extensive review of the literature.

In our work the polarimetric procedure of Hopkins has been studied in some detail, and results obtained by this procedure have been compared with those obtained by the diastase and direct acid hydrolysis methods on pure starches. The polarimetric procedure was applied to corn and wheat, using the factors found in a study of pure starches, and the results on a large number of samples were compared with those by the diastase method. A pretreatment of the ground grains was developed which removed interfering materials. Satisfactory agreement

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between the polarimetric and diastase methods was obtained with corn and wheat by this means.

### Experimental

*Starches.* The use of pure starch as a standard for evaluating analytical methods became justifiable only after a satisfactory method was developed for determining the true moisture content of this material. Water forms by far the largest nonstarch constituent, so that even a minor error in its estimation is of importance. The methods for moisture described by Sair and Fetzer (1942) were shown to be accurate within a few tenths of 1%. Other possible nonstarch constituents are fatty materials, protein, ash, and pentosans.

Table I describes the seven starches used in this work and gives

TABLE I  
STARCH SAMPLES USED

No.	Type	Preparation	Moisture content
			%
1	Corn	Commercial	11.67
2	Corn	Commercial	11.08
3	Waxy corn	Laboratory Pilot Plant from Iowa 939	12.02
4	Wheat	Laboratory Pilot Plant from Dawson	12.10
5	Wheat	Laboratory from Turkey	9.88
6	Tapioca	Commercial	12.12
7	Potato	Commercial	13.43

results for moisture by the toluene distillation or vacuum oven methods of Sair and Fetzer. The results for other nonstarch constituents shown in Table II are the averages of closely agreeing duplicate or triplicate determinations. Official methods of the Association of Official Agricultural Chemists were followed where possible. Ashing was carried out at 700°C for 3 hr. The air-dried starches were extracted for 48 hr in a Soxhlet apparatus with absolute methanol. In determining the fatty acid left on acid hydrolysis, 10 g of starch were heated on the boiling water bath for 2 hr in 220 ml of 0.7*N* hydrochloric acid. The fatty acid in the residue from acid hydrolysis was extracted with petroleum ether, titrated with alkali, and calculated as oleic acid. After ashing with magnesium nitrate, phosphorus was determined colorimetrically, using stannous chloride as the reducing agent. Pentosans were determined as furfural by distillation with 12% hydrochloric acid, as in the A.O.A.C. method, but, because pure glucose yields products which give a precipitate with phloroglucinol when this procedure is used, the furfural produced was determined with thiobarbituric acid (Bailey, 1937) after a redistillation to eliminate interfering

TABLE II  
NONSTARCH CONSTITUENTS (DRY BASIS)

Constituents	Sample						
	Corn	Corn	Waxy corn	Wheat	Wheat	Tap- ioca	Po- tato
	1	2	3	4	5	6	7
	%	%	%	%	%	%	%
Nitrogen	.089	.043	.033	.040	.051	.008	.012
Protein (N $\times$ 6.25)	.56	.27	.20	.25	.32	.05	.08
Ash	.10	.08	.07	.13	.14	.10	.32
Fatty acid (calculated as oleic, from titration of acid hydrolysis residue)	.68	.67	.08	.49	.51	.12	.01
Pentosans	—	.0	—	—	.0	—	.0
Phosphorus	.019	.016	.011	.048	.059	.011	.087
Methanol extract	.77	.75	.26	.39	.37	.22	.04
Fatty acid in extract (by titration)	—	.49	.09	.13	.11	.07	.02
Nitrogen in extract	.016	.007	.007	.008	.007	.001	.000
Phosphorus in extract	.005	.007	.001	.022	—	.001	.002
Fatty acid in residue from extraction	—	.04	—	—	.32	—	—
Phosphorus in residue from extraction	.015	.010	.007	.033	.048	.008	.082

substances. Unpublished work of this laboratory, as well as the work of others (Launer and Wilson, 1939), has shown that this procedure is necessary to eliminate interfering substances, such as hydroxymethyl furfural.

The differences between the methanol extract and the fatty acid found in the residue from acid hydrolysis are insignificant in calculating the sum of all nonstarch constituents. Except in the case of the wheat starches, the amount of methanol extract is always the greater, indicating that other substances besides fatty acid are extracted. The assumptions have been made in calculating the nonstarch constituents that all of the nitrogen in the starch is from protein, that all of the phosphorus is a part of the starch structure, and that all of the free fatty acid found on acid hydrolysis is a nonstarch constituent. Other assumptions of equal probability may be made, but because of the high purity of these starches, the figures for total percentage of starch would not be appreciably altered by these other assumptions.

The figures for nonstarch constituents and starch by difference are given in Table III. In Table IV are shown the starch results obtained by four methods. In making these determinations, 70% instead of 10% alcohol was used to remove sugars; a malt syrup of high diastatic power was used in place of the malt extract; the Scales method (Isbell, 1940) was used for sugar; and the factor 0.92 was used in place of 0.90



TABLE III  
SPECIFIC ROTATION OF STARCH

Determination	Sample						
	Corn	Corn	Waxy corn	Wheat	Wheat	Tapioca	Potato
	1	2	3	4	5	6	7
Fatty acid by acid hydrolysis, %	.68	.67	.08	.49	.51	.12	.01
Protein ( $N \times 6.25$ ), %	.56	.27	.20	.25	.32	.05	.08
Ash, %	.10	.08	.07	.13	.14	.10	.32
Total nonstarch, %	1.34	1.02	.35	.87	.97	.27	.41
Starch by difference, %	98.66	98.98	99.65	99.13	99.03	99.73	99.59
Observed rotation, $CaCl_2$ soln, 1-dm tube, 2-g sample in 100 ml	4.016	4.010	4.062	4.012	3.985	4.060	4.058
Specific rotation $[\alpha]_D$ , °	203.5	202.6	203.8	202.4	201.2	203.5	203.7

TABLE IV  
COMPARISON OF METHODS OF DETERMINING STARCH

Method	Sample						
	Corn	Corn	Waxy corn	Wheat	Wheat	Tapioca	Potato
	1	2	3	4	5	6	7
	%	%	%	%	%	%	%
Starch by difference	98.7	99.0	99.6	99.1	99.0	99.7	99.6
Starch by polarimeter ( $[\alpha]_D = 203^\circ$ )	98.9	98.8	99.8	98.8	98.2	100.0	99.9
Starch by acid hydrolysis (factor 0.92)	97.2	97.8	98.6	97.6	97.9	98.9	99.3
Starch by diastase (factor 0.92)	97.4	98.5	99.7	98.8	98.5	99.8	99.8

in converting from sugar to starch. The factor 0.92 was derived by treating pure dextrose (National Bureau of Standards' No. 41) with acid under the conditions used in both the direct acid hydrolysis and diastase methods. The use of the factor 0.93 instead of 0.90 has been suggested by Noyes (1904), Etheredge (1941), and many others. The agreement shown in Table IV between the starch by difference and the starch by diastase further supports the factor 0.92. The rotations of the starches were measured by reading the rotation of the dispersion of 2 g of starch in 100 ml of the calcium chloride solution described later, using a 1-dm tube. The specific rotations,  $[\alpha]_D$ , of the starches were calculated from the starch contents as determined by difference and the observed rotations. The average specific rotation of  $203^\circ$  was used in all further work, since the differences shown by the various kinds of starch do not exceed the possible experimental error. The

specific rotation calculated for the two wheat starches is slightly lower than  $203^\circ$ , but until a larger number of starches are studied this average value is recommended. This value of  $203^\circ$  is slightly higher than the commonly used value of  $200^\circ$  (Mannich-Lenz, 1920).

The agreement between the figures for starch determined by diastase and starch determined by difference was satisfactory, whereas the figure for starch by acid hydrolysis was consistently lower. The average values for the seven starches were: by difference, 99.25%;<sup>2</sup> by diastase, 98.93%; and by acid hydrolysis, 98.17%. No effort was made to depart from the official methods for the acid hydrolysis and diastase methods in order to obtain higher values.

*Corn and Wheat.* Determinations of the starch content of corn and wheat were carried out using essentially the A.O.A.C. tentative polarimetric method and the A.O.A.C. diastase method with the modifications previously described. Experiments on the fineness of grinding required to obtain a complete dispersion of the starch of these grains in calcium chloride solution showed that grinding through the  $\frac{1}{2}$ -mm screen of a Wiley mill was satisfactory. Comparable and equally satisfactory results were obtained by grinding corn on a Bauer Brothers mill (3,600 rpm attrition mill) and by grinding wheat on a Labconco mill.

Comparison of the results by the polarimetric and diastase methods on a series of 30 corn samples showed fairly satisfactory agreement. The average by the polarimetric method was 71.0% starch, and that by the diastase method (factor 0.92) was 69.9%. Although the polarimetric procedure, as described in the Journal of the Association of Official Agricultural Chemists, is much easier and faster than the diastase procedure, the modification described by Clendenning<sup>3</sup> for use on wheat flour offers a further advantage in speed. A comparison made between these two polarimetric procedures showed on 23 samples of corn an average of 69.5% starch by the A.O.A.C. procedure (using an alcohol wash) and 69.3% starch by the Clendenning procedure (in which 5.0 ml of 2.5% stannic chloride is added to precipitate proteins in place of washing). It is apparent that any sugar present in the calcium chloride dispersion does not appreciably affect the results obtained for starch.

When the diastase and polarimetric methods of the Association of Official Agricultural Chemists were used on wheat, the diastase method gave average results for starch that were 2.5% higher than those obtained by the polarimetric procedure. Differences in results by the two methods were especially marked when fractions from wheat milling,

<sup>2</sup> All results are calculated to the moisture-free basis.

<sup>3</sup> Dr. K. A. Clendenning very kindly furnished directions for his method of determining starch in wheat flour (unpublished).

such as bran and shorts, were analyzed. It was found that the use of stannic chloride or other protein precipitant was of no assistance on many samples since the solutions could not be filtered easily and were turbid after filtration. Balch and Phillips (1941), in a study of the determination of starch in sweet potatoes, found that an alkaline wash removed interfering materials. This procedure was found to work very well with wheat, and the alkaline wash was shown experimentally not to change the rotation of pure starch. The ether and alcohol washes recommended by the Association of Official Agricultural Chemists were replaced by three washes with 0.05*N* sodium hydroxide, followed by one wash with 0.5*N* acetic acid and two washes with alcohol. Using this alkaline wash in the polarimetric procedure, analyses of 43 samples of wheat grown in 1940 and 1942 gave 63.9% for the average starch content in comparison with 65.4% by the diastase procedure; this was a marked improvement over previous results.

The effect of the alkaline wash on the diastase determination was also investigated. For a group of 46 wheats grown in 1941, the average starch content was 65.1%, which was exactly the same as the average starch content by the polarimetric procedure using an alkaline wash. In making these comparisons, the average difference between duplicates on 42 samples was 0.27% for both methods, and the standard error of a single determination was 0.19% for the polarimetric and 0.27% for the diastase. The maximum difference between methods was 1.8%. On the other four samples, duplicates by the diastase method differed by more than 1% and were not included in the average since additional determinations were necessary. Three other samples, in which the ratio of starch to nitrogen differed from that expected, were rerun by the polarimetric method. On two of these samples in the repeated determinations the starch value was increased, making the ratio of starch to nitrogen more nearly that which was expected.

### Improved Polarimetric Method

The details of the final methods used for determining starch in corn and wheat are as follows:

#### Reagents

*Calcium chloride solution:* Dissolve 546 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  in distilled water and dilute to one liter. Adjust density to 1.30 at 20°C. Add glacial acetic acid to bring to pH of  $2.5 \pm 0.3$ .

*Stannic chloride solution:* Dissolve 2.5 g of  $\text{SnCl}_4 \cdot 5\text{H}_2\text{O}$  in 97.5 g of the  $\text{CaCl}_2$  solution.

*Sodium hydroxide solution:* 0.05*N*.

*Acetic acid solution:* 0.5*N*.

*Ethanol:* 70% by volume.

#### Determination

*Corn.* Grind the sample through a 0.5-mm Wiley screen, or to an equivalent fineness, and weigh 2 g into a 250-ml beaker. Add 10 ml  $\text{H}_2\text{O}$ , and stir to wet the

sample. Add 70 ml of  $\text{CaCl}_2$  solution. Cover with watch glass, and bring to a boil in 5 min on a hot plate. Boil 15 min, stirring as needed to keep the sample from sticking to the side of the beaker. The stirring will also help to prevent bumping and foaming, but it may be necessary to blow into the beaker at times to prevent foaming. Cool quickly, and transfer to a 100-ml volumetric flask with  $\text{CaCl}_2$  solution. Add 5 ml of  $\text{SnCl}_4$  solution and fill to the mark with  $\text{CaCl}_2$  solution. Mix well, and filter on a folded No. 2 Whatman filter paper, discarding the first 15 ml of the filtrate. Collect an additional portion of the filtrate in a separate receiver and polarize in a 10-cm tube.

Calculate the starch content as follows:

$$\text{Percentage of starch} = \frac{A \times 100 \times 100}{203 \times S}$$

where

$A$  = observed rotation,

$S$  = sample weight.

*Wheat.* Grind the sample through the Wiley 0.5-mm screen and weigh 2 g into a round-bottomed, 50-ml, heavy-duty, centrifuge tube. Add 10 ml of 0.05*N* NaOH and stir thoroughly. Centrifuge, decant, and wash twice more with alkali. Similarly, wash once with 0.5*N* acetic acid and twice with 70% alcohol. Transfer to a 250-ml beaker with 10 ml of water; add 70 ml  $\text{CaCl}_2$  solution; and disperse as for corn. Cool quickly; transfer to a 100-ml volumetric flask; and fill to the mark with  $\text{CaCl}_2$  solution. Mix well, and filter on a folded No. 4 Whatman filter paper (12.5 cm), discarding the first 15 ml of the filtrate. Complete as for corn.

### Discussion

The speed with which determinations may be completed is one of the greatest advantages of the polarimetric method. Samples may be ground, weighed, washed, dispersed, and completely analyzed within 13½ hr. A total of eight hours is required to complete a determination by the official diastase method. Over a period of days, one analyst can complete in an 8-hr working day, 14 polarimetric determinations or 10 diastase determinations on wheat using the alkaline wash, and 24 or 16, respectively, on corn using the stannic chloride precipitation in the polarimetric and the alcohol wash in the diastase method.

The differences between the proteins, starches, and pentosans of corn and wheat are responsible for the different pretreatments used for these two grains. The corn could not be washed with alkali without a loss of starch in the wash waters, as shown by an iodine test. With wheat, no loss of starch has been observed. After the addition of stannic chloride to wheat, the solution could not be filtered satisfactorily, and the filtrate was frequently too cloudy to be measured in a polarimeter. No attempt has been made to show how these differences are related to the known properties of corn and wheat proteins.

Closer agreement between the starch by difference and the starch by diastase could be obtained in the case of the seven pure starches if the factor of 0.93 were used to convert sugar to starch. This factor has been suggested by others, but, as stated previously, several experiments on pure dextrose showed that approximately 98% recovery was obtained when this substance was put through the acid hydrolysis used in the starch determination. The lower values found for the pure

starches by the direct acid hydrolysis and the diastase methods may indicate difficulty in obtaining the complete hydrolysis of starch to reducing sugars.

The good agreement obtained between the two methods—one physical and one chemical—lends support to both. The polarimetric method is empirical, since the specific rotation of starch is obtained by measurements on noncrystalline materials, and is to some extent dependent on the accuracy of analysis for the nonstarch constituents. Use of this method on many samples of corn and wheat has shown it to be satisfactory for calculating yields of starch or products from starch. Results obtained by this method are reproducible within about 1%. The accuracy of the method has not been determined, but it is believed that it gives results within 1% of the true values.

The polarimetric method may be applicable to many other grains and in this laboratory it has been used on sorghums, oats, barley, and rye. Since no study was made on pure starches from these grains, it was necessary to use the specific rotation of  $203^\circ$  found for corn and wheat. In applying the polarimetric method to other grains, special treatments, similar to the alcohol and other washes, the stannic chloride addition, or the alkaline wash, may be found necessary, but no data have been obtained on these applications to date.

### Summary

Seven samples of starch, comprising two from corn, two from wheat, and one each from waxy corn, tapioca, and potato, were analyzed for nonstarch constituents.

The specific rotation,  $[\alpha]_D$ , of all these starches was found to be  $203^\circ$  in calcium chloride dispersion, when corrected for the nonstarchy material.

Modifications of the polarimetric method for starch in corn and wheat were studied and procedures devised which, for these two grains, gave results agreeing closely with those obtained by the diastase-acid hydrolysis method. These improved polarimetric procedures are described.

### Acknowledgment

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## A NOTE ON THE COMPARATIVE EFFICIENCY OF L-ASCORBIC ACID AND POTASSIUM BROMATE AS DOUGH CONDITIONERS

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The oxidizing effect of l-ascorbic acid in bread doughs has been the subject of several investigations. Jørgensen (1935, 1939) and Feaster and Cathcart (1941) showed that l-ascorbic acid was equivalent to potassium bromate as a dough conditioner on a weight-for-weight basis. Recent experiments in this laboratory have shown that l-ascorbic acid is not quite as effective as potassium bromate as a dough conditioner.

Baking tests were conducted, using the procedure outlined in *Cereal Laboratory Methods* (4th ed., 1941). Potassium bromate and l-ascorbic acid were compared in these baking tests. Three regular commercial-run flours were used in the tests: (1) short patent flour; (2) stuffed straight flour; and (3) whole wheat flour. All of these flours were treated with Agene at the mill. The results given in Table I show that l-ascorbic acid affects the volume and improves the grain, texture, and crumb color of the loaves in a manner similar to bromate. However, all of the data show that it is necessary to use approximately one and one-half times as much l-ascorbic acid as bromate to obtain the

TABLE I

COMPARISON OF L-ASCORBIC ACID AND POTASSIUM BROMATE AS DOUGH CONDITIONERS  
(All flours treated with Agene)

Quantities given in milligrams per 100 grams of flour

	Loaf vol- ume	Grain	Texture	Crumb color
Short patent flour				
Control	680	Slightly elongated; slightly attenuated	Soft	Very slightly creamy
1.0 mg $\text{KBrO}_3$	660	Improved attenuation	Soft	Creamy white
1.0 mg l-ascorbic acid	680	Slightly improved attenuation	Soft	Shade darker than bromate loaf
1.5 mg l-ascorbic acid	665	Improved attenuation	Soft plus	Shade brighter than bromate loaf
2.0 mg l-ascorbic acid	675	Improved attenuation	Soft plus	Shade brighter than bromate loaf
Stuffed straight flour				
Control	655	Coarse, poor attenua- tion	Soft minus	Creamy and dull
1.0 mg $\text{KBrO}_3$	705	Slightly elongated; much improved attenuation	Soft plus	Much brighter than control
1.0 mg l-ascorbic acid	665	Coarse; slightly im- proved attenuation	Soft	Shade brighter than control
1.5 mg l-ascorbic acid	695	Slightly elongated; im- proved attenuation	Soft plus	Much brighter than control
2.0 mg l-ascorbic acid	680	Slightly elongated; im- proved attenuation	Soft plus	Much brighter than control
Whole wheat flour				
Control	560	Coarse	Harsh	Satisfactory
2.0 mg $\text{KBrO}_3$	625	Improved attenuation	*	Much brighter than control
2.0 mg l-ascorbic acid	590	Slightly improved attenuation	**	Shade brighter than control
3.0 mg l-ascorbic acid	620	Improved attenuation	*	Much brighter than control
4.0 mg l-ascorbic acid	590	Improved attenuation	*	Much brighter than control

\* Much improved over control.

\*\* Slightly improved over control.

same results. This was verified by other experiments in the present study. During the course of the experiments, d-iso-ascorbic acid was found to be ineffective as a dough conditioner, confirming earlier work by Feaster and Cathcart (1941).

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## BOOK REVIEWS

**The Chemistry and Technology of Food and Food Products. Volume I.** Prepared by a group of specialists under the editorship of Morris B. Jacobs. 952 pp. Interscience Publishers, Inc., New York, N. Y. 1944. Price \$10.50.

This book is the first of two volumes designed to survey the fields of food chemistry and technology. It includes the contributions of 21 collaborators selected for their qualifications to present an authoritative discussion of the many aspects of this subject. Divided into two parts, "Fundamentals" and "Foods," this first volume is devoted mainly to the chemistry of foods and will be followed shortly by the second volume in which the technological aspects will be emphasized.

Ten subjects are considered in the section dealing with "Fundamentals." These are: (1) The Physical Chemistry of Foods (St. John), (2) The Carbohydrates (Degering), (3) Lipids (Baizer and Zahnd), (4) Some Aspects of the Chemistry of Amino Acids in Proteins (Zahnd and Baizer), (5) Enzymes (Stern), (6) Vitamins, Vitagens, and Hormones (Rosenberg), (7) Mineral Matters and Other Inorganic Food Adjuncts (Carr), (8) Coloring Matters in Foods (Jablonski), (9) The Digestion and Fate of Foodstuffs (Wilhelmi), and (10) Food Spoilage and Food Poisoning (Halvorsen).

This is an imposing array of titles and indicates the intent of the editor to present a truly comprehensive treatise. In some instances, however, the allotted space is inadequate for the subject material, and important omissions will be noted. The discussion of physical chemistry is essentially a series of definitions, while the chapter dealing with carbohydrates will be of limited value to the reader seeking information about dextrins and starches. The amino acids and proteins are discussed more extensively and include references to recent literature of considerable importance to the chemistry of foods. Lipids are rather sketchily considered, and the subject of enzymes pays little attention to practical applications. Rancidity is barely mentioned, and it is hoped that Volume II will include a discussion of this important phenomenon. Many food technologists will want to supplement these chapters with other sources of information. Vitamins are reviewed almost entirely from the purely chemical standpoint, with no reference to the phases of methodology so important to many workers in food fields. This chapter is essentially a condensation of the book recently written by the same author. The chapters dealing with minerals, food colors, digestive processes, and food spoilage are of general interest. While brief, they cover the salient points prerequisite to an appreciation of food problems.

Part II, entitled "Foods," presents discussions of 12 food fields. Here the reader will find specific information relating to: (1) Milk, Cream, and Dairy Products (Jacobs), (2) Meat and Meat Products (Urban), (3) Fish, Shell Fish, Crustacea (Stansby), (4) Poultry and Eggs (Pennington), (5) Edible Oils and Fats (Bailey), (6) Cereal Grains (Geddes), (7) Baking and Bakery Products (Cathcart), (8) Vegetables, Mushrooms, Nuts, and Fruits (Lee), (9) Carbohydrate and Sugar Foods (Degering), (10) Confectionery and Cacao Products (Schoen), (11) Coffee and Tea (Ukers), and (12) Flavors, Spices, and Condiments (Worrell).

The subject of dairy products describes some of the physical and physiochemical properties of milk and 17 types of milk products. In many instances, the treatment is rather brief. Numerous tables carry information about the composition of dairy products. The subject of nutrition is rather incomplete, less than half a page being devoted to the vitamins. Considerable information has been obtained since 1938, the most recent source of information presented. Of greatest interest to the cereal chemist will be the chapters dealing with cereal grains and bakery products. These two chapters comprise nearly one-sixth of the volume, reflecting the extensive treatment given these items. The chapter on cereal grains includes considerations of production, structure, classification, and chemical composition. Barley, buckwheat,



corn, oats, rice, rye, and wheat are separately treated. Brief consideration is also given to cottonseed, peanut, and soybean flours. The chapter on baking and bakery products describes the ingredients and reactions taking place in the production of bread, cakes, cookies, etc. Such subjects as staleness, nutritive properties, and microbiology of bakery products are included. The chapter dealing with carbohydrate and sugar foods is out of place in the general plan of the book. It is an extension of the chapter by the same author appearing under "Fundamentals" and the reason for its inclusion under "Foods" is rather obscure. Those seeking knowledge about the practical aspects of carbohydrate foods will be disappointed.

An evaluation of the first volume with respect to its utility is made somewhat difficult by the type of arrangements planned for the two-volume set. In many instances, a discussion of the chemistry and technology of foods should go hand in hand, and the differentiation placing one phase in one volume and the second in another has certain disadvantages. The appearance of brevity in many of the chapters may be removed by the treatment in the second volume. This does not remove, however, the rather disconcerting situation as illustrated in the chapter on Confectionery and Cacao Products, where definitions for cacao, cocoa, and chocolate are presented, together with the statement that standards for different cacao products are discussed in Volume II, Chapter 18. From the convenience standpoint, it would have seemed preferable to discuss one subject completely, rather than attempt a rather indefinite segregation in two volumes.

Despite many of the limitations, the editor and collaborators are to be congratulated on their attempt to consolidate the many aspects of food chemistry and technology. Many of the chapters are well referenced, and any information not found in the descriptive parts can be sought conveniently by reference to the selected bibliography. The present volume, together with the one which is expected to appear shortly, should occupy a prominent place in the library of all interested in foods.

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**Chemistry and Industry of Starch.** Edited by Ralph W. Kerr. 472 pp. Academic Press, Inc., New York, N. Y. 1944. Price \$8.50.

The editor of this book, in which the accumulated knowledge of starch and products derived therefrom is brought up to date, is assisted by 14 co-authors, each a specialist in some phase of starch chemistry. Greater emphasis is given the American viewpoint and practice of technology than was done in most earlier books in this field. The text is not intended to be comprehensive, since a single volume scarcely offers sufficient space to do this with the many divisions of the subject. The editor expresses the hope that this survey "will be of benefit not only to the carbohydrate chemist but to the technologist, whose daily task is concerned with more efficient production of starch products and with their more efficient utilization."

The subject matter is contained in five sections comprising 22 chapters. The section outline which follows shows the order of treatment of the various topics and indicates the scope of the text.

Section I. Occurrence in nature—source of starch; size and shape of starch granules; the hilum; striations; swelling of granules in water; description of individual starches; photomicrographs of various starches.

Section II. Preparation—the manufacture of corn, modified corn, tapioca, wheat, white potato, sweet potato, arrowroot and sago starches; evaluation of modified starches in practice.

Section III. Properties—physical and chemical properties of starch (composition and structure).

Section IV. Reactions—the hydrogen bond in starch as a basis for interpreting its behavior and reactivity; derivatives of starch; oxidation of starch; dextrinization; manufacture of dextrans; acid hydrolysis of starch; the amylases (properties and production); modification of starch by enzymes; miscellaneous reactions (iodine, formaldehyde, other aldehydes, hydrogenation).

Section V. Uses—uses of starch in paper manufacture; uses of starch and starch products in the fermentation industries; use of starch and starch products in foods; use of starch products in the textile industry; starch adhesives.

The text maintains a good balance between fundamental and applied knowledge and should inspire more fundamental research leading to new industrial applications of starch. Author and subject indices are included.

The editor, authors, and publishers are to be congratulated in providing this timely text on starch which will be a valuable reference work for everyone interested in carbohydrate chemistry.

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**Micromeritics.** J. M. DallaValle. 428 pp. Pitman Publishing Company, New York, N. Y. 1943. Price \$8.50.

The author has coined the word "micromeritics" to designate the technology of fine particles, the term "fine" being defined to cover the range of particle diameters from  $10^{-1}$  to  $10^5 \mu$  ( $10^{-4}$  to 100 mm). To quote the author, "This text is intended as a guide to the general subject of the behavior and characteristics of small particles. It brings together a mass of widely scattered information on methods of particle-measurement, size-distributions, packing arrangements, and a general theory concerning the physical properties of finely divided substances." For this reason alone, it will probably be a welcome addition to the technologist's reference library.

The subjects covered range from a rigorous mathematical treatment of the dynamics of small particles through discussions on their electrical, optical, sonic, chemical, and thermodynamic properties to a dissertation on muds and slurries. Because of the broad nature of the subject covered, the book assumes somewhat of an incoherent nature and, for this reason, will find its greatest acceptance as a reference rather than as a textbook.

It is felt that many of the mathematical presentations are "thrown" at the reader without proper introduction and explanation; for instance, the equations of motion of a particle given on page 19 are simply stated to be the equations of motion in two dimensions. Not until the reader has inspected the equations does he learn that they are those for the special case in which one of the dimensions is the vertical, and the particle is subject to the force due to gravity. Only after further inspection is it revealed that the motion is in a medium in which the density is different from zero. Perhaps this criticism should be overlooked in view of the volume of material covered, but improper presentations of this kind become confusing.

Because of the author's election to treat the subject in a rigorous manner, the student who has had no more than an "exposure" to higher mathematics will probably find himself beyond his depth on numerous occasions.

The 38-page bibliography at the end of the book will be well accepted.

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# INDEX TO VOLUME XXI

## AUTHORS

	PAGE		PAGE
Aitken, T. R., M. H. Fisher, and J. A. Anderson. Effect of protein content and grade on farinograms, extensograms, and alveograms.....	465	Combs, Y. F. An instrument for determining the compressibility and resistance to shear of baked products.....	319
—, —, and — Reproducibility studies and some effects of technique on extensograms and alveograms.....	489	Cox, M. J., M. M. MacMasters, and G. E. Hilbert. Effect of the sulfurous acid steep in corn wet milling.....	447
— (See B. N. Smallman).....	499	Davis, C. F. (See Stephen Laufer)....	267
Alcock, A. W. (See A. Hoffer) 210, 515,	524	Davis, H. A. (See R. J. Dimler).....	430
Anderson, G. (See R. B. Meckel).....	280	Dickson, A. D. (See W. J. Olson).....	533
Anderson, J. A., K. W. Neathy, and I. Levi. A preharvest survey of the protein content of western Canadian wheat of the 1943 crop.....	283	Dimler, R. J., H. A. Davis, C. E. Rist, and G. E. Hilbert. Production of starch from wheat and other cereal flours.....	430
— (See T. R. Aitken).....	465, 489	Earle, F. R., and R. T. Milner. Improvements in the determination of starch in corn and wheat.....	567
— (See W. J. Eva).....	560	Edelmann, E. C., and W. H. Cathcart. The measurement of oven spring as an aid in controlling flour quality.....	385
— (See W. O. S. Meredith).....	233	— (See W. H. Cathcart).....	575
Andrews, A. C. (See C. O. Swanson)....	140	Elion, E. Correlation between chemical structure of some organic compounds and their action as flour improvers and as inhibitors of proteolytic enzymes....	314
Andrews, J. S. Book review: The chemistry and technology of food and food products, ed. by M. B. Jacobs.....	577	Elledge, Muriel. (See R. H. Harris)....	38
— Report of the 1943-44 methods of analysis subcommittee on riboflavin assay.....	398	Eva, W. J., and J. A. Anderson. The relations between Kjeldahl protein, Zeleny protein, and loaf volume in western Canadian wheats and flours... ..	560
— Report of the 1943-44 methods of analysis subcommittee on thiamine assay.....	388	— (See W. O. S. Meredith).....	233
Anker, C. A., and W. F. Geddes. Gelatinization studies upon wheat and other starches with the amylograph....	335	Evans, J. W. Book review: Chemistry and industry of starch, ed. by R. W. Kerr.....	578
Ausemus, E. R. (See P. P. Merritt).....	199	Evans, Ruth. (See W. J. Olson).....	533
Bailey, C. H. Book review: Physical properties of dough, by C. O. Swanson	79	Fieger, E. A. (See V. R. Williams).....	540
Baker, J. C., H. K. Parker, and M. D. Mize. The action of oxidizing agents on sulfhydryl compounds in dough....	97	Finney, K. F., and M. A. Barmore. Flour blends and the question of complementary effects.....	65
Balls, A. K., and T. H. Harris. The inhibitory effect of a protamine from wheat flour on the fermentation of wheat mashies.....	74	Fisher, M. H. (See T. R. Aitken)....	465, 489
Barmore, M. A. (See K. F. Finney).....	65	Fortmann, Karl. (See R. M. Sandstedt)	172
Bauernfeind, J. C., M. B. Smith, J. C. Garey, Werner Baumgarten, F. H. Gustoff, and Leonard Stone. Nutrient content of alcohol fermentation by-products from various grains.....	421	Freilich, J., and C. N. Frey. Dough oxidation and mixing studies. VI. Effects on oxidizing agents in the presence of reducing matter.....	241
Baumgarten, Werner. (See J. C. Bauernfeind).....	421	Frey, C. N. (See J. Freilich).....	241
Bayfield, E. G. (See H. D. Young).....	149	Garey, J. C. (See J. C. Bauernfeind)....	421
Blish, M. J. Book review: The constituents of wheat and wheat products, by C. H. Bailey.....	334	Geddes, W. F. Book review: Cellulose and cellulose derivatives, ed. by Emil Ott.....	333
Bode, C. E. (See V. H. Morris).....	49	— (See C. A. Anker).....	335
Borchardt, L. F. Book review: Micromeritics, by J. M. DallaValle.....	579	— (See A. Hoffer).....	210, 515, 524
Brown, R. O., and C. G. Harrel. The use of the amylograph in the cereal laboratory.....	360	Glick, David. Simplified procedures for the determination of thiamine in wheat flours and bread by the thiochrome method.....	119
Carlin, G. T. A microscopic study of the behavior of fats in cake batters....	189	Gortner, R. A., and R. T. MacDonald. Studies on the fractionation of zein... ..	324
Cathcart, W. H., and E. C. Edelmann. A note on the comparative efficiency of l-ascorbic acid and potassium bromate as dough conditioners.....	575	Goulden, C. H. Experimental design for cereal chemists.....	159
— (See E. C. Edelmann).....	385	Gustoff, F. H. (See J. C. Bauernfeind)....	421
		Hanks, William. (See E. A. Vaupe)....	16
		Harrel, C. G. (See R. O. Brown).....	360

- (See Howard Lincoln) . . . . . 274
- Harris, R. H., L. D. Sibbitt, and Muriel Elledge.** Varietal station and seasonal effects upon some properties of mixograms made from hard red spring wheat flours by various mixing methods. . . . . 38
- , —, and **G. M. Scott.** The effect of temperature differences on some mixogram properties of hard red spring wheat flours. . . . . 374
- Harris, T. H.** (See A. K. Balls) . . . . . 74
- Heizer, H. K.** (See V. H. Morris) . . . . . 49
- Hilbert, G. E.** (See M. J. Cox) . . . . . 447
- (See R. J. Dimler) . . . . . 430
- (See M. M. MacMasters) . . . . . 258, 548
- Hildebrand, F. C., and R. C. Koehn.** Sources of error in the determination of the protein content of bulk wheat. . . . . 370
- Hlynka, K.** (See W. O. S. Meredith) . . . . . 251
- Hoffer, A., A. W. Alcock, and W. F. Geddes.** The effect of variations in Canadian spring wheat on the thiamine and ash of long extraction flours. . . . . 210
- , —, and —. A rapid method for the determination of riboflavin in wheat and wheat products. . . . . 524
- , —, and —. Some factors affecting the determination of riboflavin by the fluorometric method. . . . . 515
- Hove, E. L.** (See Howard Lincoln) . . . . . 274
- Howe, M.** Report of the 1943-44 methods of analysis subcommittee on the determination of iron in cereal products . . . . . 412
- Johnson, J. A.** (See C. O. Swanson) . . . . . 222
- Kik, M. C., and F. B. Van Ledingham.** Nicotinic acid in products of commercial rice milling and in rice varieties. . . . . 154
- Kneen, Eric.** A comparative study of the development of amylases in germinating cereals. . . . . 304
- Koehn, R. C.** (See F. C. Hildebrand) . . . . . 370
- Kretovich, V. L.** Biochemistry of the damage to grain by the wheat-bug. . . . . 1
- Lamb, C. A.** Sifted wheat meal mixograms for selecting soft wheat varieties . . . . . 57
- Laufer, Stephen, Henry Tauber, and C. F. Davis.** The amylolytic and proteolytic activity of soybean seed. . . . . 267
- Levi, I.** (See J. A. Anderson) . . . . . 283
- Lincoln, Howard, E. L. Hove, and C. G. Harrel.** The loss of thiamine on cooking breakfast cereals. . . . . 274
- Loving, H. J.** (See F. R. Schuwin) . . . . . 27
- Lyman, J. F.** (See M. R. Shetlar) . . . . . 295
- MacDonald, R. T.** (See R. A. Gortner) . . . . . 324
- McKim, Elizabeth.** Report of the 1943-44 committee on self-rising and phosphated flours. . . . . 419
- MacMasters, M. M., and G. E. Hilbert.** The composition of the "amyloextrin" fraction of wheat flour. . . . . 548
- , —, and —. Stored and damaged wheats for starch production. . . . . 258
- (See M. J. Cox) . . . . . 447
- Meckel, R. B., and G. Anderson.** Thiamine retention in the commercial production of zwieback. . . . . 280
- Meredith, W. O. S., W. J. Eva, and J. A. Anderson.** Effect of variety and environment on some qualities of malted wheat flour. . . . . 233
- , **K. Hlynka, and H. R. Sallans.** Laboratory malting. IV. Germination chamber for routine malting tests . . . . . 251
- Merritt, P. P., and E. R. Ausemus.** Effect of scab on the quality of hard red spring wheat. . . . . 199
- Milner, R. T.** (See F. R. Earle) . . . . . 567
- Mize, M. D.** (See J. C. Baker) . . . . . 97
- Morris, V. H., C. E. Bode, and H. K. Heizer.** The use of the mixogram in evaluating quality in soft wheat varieties. . . . . 49
- Myers, H. S., and E. B. Working.** The sulfhydryl groups of wheat flour. . . . . 32
- Neatby, K. W.** (See J. A. Anderson) . . . . . 283
- Olson, W. J., Ruth Evans, and A. D. Dickson.** A modification of the Kneen and Sandstedt methods for the determination of alpha- and beta-amylases in barley malt. . . . . 533
- Parker, H. K.** (See J. C. Baker) . . . . . 97
- Pratt, D. B.** Report of the 1943-44 committee on relation of laboratory baking to shop practice. . . . . 556
- Rist, C. E.** (See R. J. Dimler) . . . . . 430
- Sallans, H. R.** (See W. O. S. Meredith) . . . . . 251
- Sandstedt, R. M., and Karl Fortmann.** Effect of environment during the growth and development of wheat on the baking properties of its flour. . . . . 172
- Schiller, G. W.** Simplification of the thiochrome method for thiamine determination. . . . . 544
- Schuwin, F. R.** Report of the 1943-44 committee on testing biscuit and cracker flours. . . . . 407
- , and **H. J. Loving.** The shortener tolerance of biscuit and self-rising flours. . . . . 27
- Scott, G. M.** (See R. H. Harris) . . . . . 374
- Shetlar, M. R., and J. F. Lyman.** Effect of bran on bread baking. . . . . 295
- Sibbitt, L. D.** (See R. H. Harris) . . . . . 38, 374
- Smallman, B. N., and T. R. Aitken.** Susceptibility of biscuits to insect damage. . . . . 499
- Smith, M. B.** (See J. C. Bauernfeind) . . . . . 421
- Stern, Rosa.** Effects on flour doughs of extracts from unfermented and fermented wheat germ. . . . . 81
- A note on the presence of fiber in the "amyloextrin" fraction of wheat flour. . . . . 366
- Stone, Leonard.** (See J. C. Bauernfeind) . . . . . 41
- Swanson, C. O.** Factors that influence the physical and other properties of wheat. V. Effect of frequent rains accompanied by storms on Blackhall, Chielkan, and Tenmarq. . . . . 126

- and A. C. Andrews. Factors which influence the physical properties of dough. VI. Effect of cysteine and some other substances on mixogram patterns. . . . . 140
- and J. A. Johnson. The effect of some wetting and reducing agents on the mixing time and on the quality of bread. . . . . 222
- Tauber, Henry. (See Stephen Laufer). 267
- Tremain, H. E. The relative germicidal effectiveness of certain chlorine compounds on the thermoduric bacterial spores of wheat. . . . . 511
- Van Landingham, F. B. (See M. C. Kik). . . . . 154
- Vaupel, E. A., and William Hanks. pH variations in soda crackers and their control with diammonium phosphate. 16
- Williams, V. R., and E. A. Fieger. A study of some of the vitamin B-complex factors in rice and its milled products, II. . . . . 540
- Working, E. B. (See H. S. Myers). . . . . 32
- Worzella, W. W. The effect of level of soil fertility on wheat quality. . . . . 107
- Young, H. D., and E. G. Bayfield. Effect of hydrocyanic acid on the baking quality of flour. . . . . 149

## SUBJECTS

- Aerosol, Effect on dough (Swanson and Andrews). . . . . 140
- Alcohol fermentation, Nutrient content of by-products (Bauernfeind, Smith, Garey, Baumgarten, Gustoff, and Stone). . . . . 421
- Alpha amylase, see Amylases
- Alveograms
- Effect of protein content and grade on (Aitken, Fisher, and Anderson). . . . . 465
- Reproducibility and effects of technique on (Aitken, Fisher, and Anderson). . . . . 489
- Amylases
- Cereal, development in germinating (Kneen). . . . . 304
- Malt, modification of Kneen and Sandstedt method for (Olson, Evans, and Dickson). . . . . 533
- Soybean, activity (Laufer, Tauber, and Davis). . . . . 267
- Amylodextrin
- Composition in flour (MacMasters and Hilbert). . . . . 548
- Fiber in wheat flour fraction (Stern). . . . . 369
- Amylograph
- Use in cereal investigations (Brown and Harrel). . . . . 360
- Use in wheat starch gelatinization studies (Anker and Geddes). . . . . 335
- Analysis, Relation to experimental design (Goulden). . . . . 159
- Ascorbic acid, L. Efficiency as dough conditioner (Cathcart and Edelmann). . . . . 575
- Ash, Relation to thiamine content of flour (Hoffer, Alcock, and Geddes). . . . . 210
- Bacteria, Sporulating thermoduric, effect of chlorine compounds on in wheat (Tremain). . . . . 511
- Baked products, Instrument to measure compressibility and resistance to shear (Combs). . . . . 319
- Baking
- Effect of bran in bread (Shetlar and Lyman). . . . . 295
- Tests, correlation with shop practice (Pratt). . . . . 556
- Thiamine loss in zwieback (Meckel and Anderson). . . . . 280
- Barley malt, see Malt
- Beta amylase, see Amylases
- Biotin, In rice varieties and milling products (Williams and Fieger). . . . . 540
- Biscuit flour
- Shortening tolerance (Schwain and Loving). . . . . 27
- Testing (Schwain). . . . . 407
- Biscuits, Susceptibility to insect damage (Smallman and Aitken). . . . . 499
- Book reviews
- Cellulose and cellulose derivatives, ed. by Emil Ott (Geddes). . . . . 333
- Chemistry and industry of starch, ed. by R. W. Kerr (Evans). . . . . 578
- The chemistry and technology of food and food products, ed. by M. B. Jacobs (Andrews). . . . . 577
- The constituents of wheat and wheat products, by C. H. Bailey (Blish). . . . . 334
- Micromeritics, by J. M. DallaValle (Borchardt). . . . . 579
- Physical properties of dough, by C. O. Swanson (Bailey). . . . . 79
- Bran, Effect on bread baking (Shetlar and Lyman). . . . . 295
- Bread
- Color, effect of flour blending (Finney and Barmore). . . . . 65
- Effect of bran in baking (Shetlar and Lyman). . . . . 295
- Improvers, efficiency of L-ascorbic acid and potassium bromate (Cathcart and Edelmann). . . . . 575
- Iron in, determination (Howe). . . . . 412
- Loaf volume, see Loaf volume
- Quality, effect of wetting and reducing agents (Swanson and Johnson). . . . . 222
- Riboflavin in, by fluorometric assay (Andrews). . . . . 398
- Thiamine in, by thiocchrome assay (Andrews). . . . . 388
- (Glick). . . . . 119
- Breakfast cereals, see Cereals, Breakfast
- Cake batter, Microscopic study of fats in (Carlin). . . . . 189
- Cereal amylases, see Amylases, Cereal
- Cereal chemists, Experimental design for (Goulden). . . . . 159

Cereal starch, see Starch, Cereal

**Cereals**

- Breakfast, thiamine loss on cooking (Lincoln, Hove, and Harrel)..... 274
- Germinating, amylase development in (Kneen)..... 304

**Cereals and cereal products**

- Amylograph in testing (Brown and Harrel)..... 360
- Iron determination in (Howe)..... 412
- Thiamine assay simplification (Schiller)..... 544

**Chloramine**, Germicidal action on thermophilic bacterial spores of wheat (Tremain)..... 511

**Chlorine compounds**, Germicidal action on thermophilic bacterial spores of wheat (Tremain)..... 511

**Collaborative studies**

- Biscuit and cracker flour testing (Schwain)..... 407
- Iron in cereal products (Howe)..... 412
- Relation of laboratory baking to shop practice (Pratt)..... 556
- Riboflavin assays (Andrews)..... 398
- Thiamine assays (Andrews)..... 388

**Committee reports**

- Committee on relation of laboratory baking to shop practice, 1943-44 (Pratt)..... 556
- Committee on self-rising and phosphated flours, 1943-44 (McKim)..... 419
- Committee on testing biscuit and cracker flours, 1943-44 (Schwain)..... 407
- Methods of analysis, subcommittee, 1943-44 (Andrews)..... 388, 412
- (Howe)..... 412

**Compressibility** of baked products, instrument for determining (Combs)..... 319

**Cooking**, Thiamine loss in breakfast cereals (Lincoln, Hove, and Harrel)..... 274

**Cooky flour**, Testing (Schwain)..... 407

**Corn**

- Starch, see Starch, Corn
- Wet milling, effect of sulfurous acid steep (Cox, MacMasters, and Hilbert)..... 447

**Cracker flour**, Testing (Schwain)..... 407

**Crackers**, pH variation and control (Vaupel and Hanks)..... 16

**Cysteine**, Effect on mixogram patterns (Swanson and Andrews)..... 140

**Design**, Experimental for cereal chemists (Goulden)..... 159

**Diammonium phosphate**, pH control in crackers with (Vaupel and Hanks)..... 16

**Dough**

- Conditioners, efficiency of *L*-ascorbic acid and potassium bromate (Cathcart and Edelmann)..... 575
- Effect of
  - extracts from fermented and unfermented wheat germ (Stern)..... 81
  - oxidizing agents in presence of reducing matter (Freilich and Frey)..... 241
  - oxidizing agents on sulfhydryl (Baker, Parker, and Mize)..... 97
  - temperature on mixograms (Harris, Sibbitt, and Scott)..... 374
- Mixing, effect of method on mixograms (Harris, Sibbitt, and Elledge)..... 38

**Dough (Continued):**

Mixing time, effect of wetting and reducing agents (Swanson and Johnson)..... 222

**Physical properties**

- correlation of tests with shop practice (Pratt)..... 556
- effect of cysteine and other substances (Swanson and Andrews)..... 140
- effect of protein content and grade (Aitken, Fisher, and Anderson)..... 465
- reproducibility and effects of technique on measurements (Aitken, Fisher, and Anderson)..... 489

**Experimental design** for cereal chemists (Goulden)..... 159

**Extensograms**

- Effect of protein content and grade on (Aitken, Fisher, and Anderson)..... 465
- Reproducibility and effects of technique on (Aitken, Fisher, and Anderson)..... 489

**Farinograms**, Effect of protein content and grade on (Aitken, Fisher, and Anderson)..... 465

**Fats**, Microscopic study of in cake batter (Carlin)..... 189

**Fermentation**

- By-products, nutrient content (Bauernfeind, Smith, Garey, Baumgarten, Gustoff, and Stone)..... 421
- Effect of flour protamine (Balls and Harris)..... 74

**Fertility** of soil, Effect on wheat quality (Worzella)..... 107

**Fiber**, In amylopectin fraction of flour (Stern)..... 369

**Flour**

- Amylopectin fraction
  - composition (MacMasters and Hilbert)..... 548
  - fiber in (Stern)..... 369
- Ash in relation to thiamine content (Hoffer, Alcock, and Geddes)..... 210

**Baking quality**

- effect of
  - flour blending (Finney and Barmore)..... 65
  - hydrocyanic acid (Young and Bayfield)..... 149
  - scab on wheat (Merritt and Ausemus)..... 199
  - wheat-bug damage (Kretovich)..... 1
  - wheat growth environment (Sandstedt and Fortmann)..... 172
  - oven spring in measuring (Edelmann and Cathcart)..... 385

**Biscuit**, see Biscuit flour

**Blends** as related to complementary effects (Finney and Barmore)..... 65

**Canadian**, relation of protein to loaf volume (Eva and Anderson)..... 560

**Cooky**, see Cooky flour

**Cracker**, see Cracker flour

**Dough**, see Dough

**Hard red spring wheat**, mixograms (Harris, Sibbitt, and Elledge)..... 38

**Improvers**, correlation between chemical structure, action, and enzyme inhibiting action (Elion)..... 314

*Flour (Continued):*

Iron in, determination (Howe).....	412
Malted, effect of variety and environment on quality (Meredith, Eva, and Anderson).....	233
Mixogram properties, effect of temperature (Harris, Sibbitt, and Scott).....	374
Phosphated, testing (McKim).....	419
Protamines, effect on wheat mash fermentation (Balls and Harris).....	74
Protein in, effect on farinograms, extensograms, and alveograms (Aitken, Fisher, and Anderson).....	465
Riboflavin in	
by fluorometric assay (Andrews)...	398
rapid fluorometric method (Hoffer, Alcock, and Geddes).....	524
sources of error in fluorometric method (Hoffer, Alcock, and Geddes).....	515
Self-rising	
shortening tolerance (Schwain and Loving).....	27
testing (McKim).....	419
Soft wheat, mixograms (Morris, Bode, and Heizer).....	49
Sulphydryl groups (Myers and Working).....	32
Thiamine in	
effect of wheat variations (Hoffer, Alcock, and Geddes).....	210
by thiochrome assay (Andrews)...	388
— (Glick).....	119
Viscosity studies with the amylograph (Brown and Harrel).....	360
Fluorometric assay for riboflavin	
In flour and bread (Andrews).....	398
Rapid method (Hoffer, Alcock, and Geddes).....	524
Sources of error in (Hoffer, Alcock, and Geddes).....	515
Fractionation of zein (Gortner and MacDonald).....	324
Fumigants, Effect on flour baking quality (Young and Bayfield).....	149
Gas retention in dough, Oven spring in measuring (Edelmann and Cathcart).....	385
Gelatinization of wheat starch (Anker and Geddes).....	335
Germination	
Chamber for routine malting tests (Meredith, Hlynka, and Sallans)...	251
Effect on amylase development in cereals (Kneen).....	304
Glutathione, Effect of oxidizing agents on in dough (Freilich and Frey)...	241
Grain fermentation by-products, Nutrient content (Bauernfeind, Smith, Garey, Baumgarten, Gustoff, and Stone).....	421
Hydrocyanic acid, Effect on flour baking quality (Young and Bayfield).....	149
Hydrogen ion concentration, Variation and control in crackers (Vaupel and Hanks).....	16
Insects, Damage to biscuits (Smallman and Aitken).....	499
Iron, In cereal products, determination (Howe).....	412

Kjeldahl protein determination, Relation to Zeleny method and loaf volume (Eva and Anderson).....	560
Kneen and Sandstedt method for amylases in barley malt, modification of (Olson, Evans, and Dickson).....	533
Loaf volume	
Effect of flour blending (Finney and Barmore).....	65
Relation to Kjeldahl and Zeleny protein determination (Eva and Anderson).....	560
Malt	
Amylases, see Amylases, Malt	
Modification of Kneen and Sandstedt method for amylases in (Olson, Evans, and Dickson).....	533
Malted flour, see Flour, Malted	
Malting, Germination chamber for routine tests (Meredith, Hlynka, and Sallans).....	251
Milling, Corn, effect of sulfurous acid in wet (Cox, MacMasters, and Hilbert).....	447
Mixing of dough, see Dough, Mixing	
Mixograms	
Effect of	
cysteine and other substances (Swanson and Andrews).....	140
temperature (Harris, Sibbitt, and Scott).....	374
variety, station, and season (Harris, Sibbitt, and Elledge).....	38
Hard red spring wheat flour (Harris, Sibbitt, and Elledge).....	3
— (Harris, Sibbitt, and Scott).....	374
Sifted soft wheat meal (Lamb).....	57
Soft wheat varieties (Morris, Bode, and Heizer).....	49
Nicotinic acid, In rice varieties and milling products (Kik and Van Landingham).....	154
Nutritive value, Fermentation by-products (Bauernfeind, Smith, Garey, Baumgarten, Gustoff, and Stone)...	421
Oven spring, Measurement in flour quality control (Edelmann and Cathcart).....	385
Oxidizing agents	
Effect on	
dough in presence of reducing matter (Freilich and Frey).....	241
sulphydryl in dough (Baker, Parker, and Mize).....	97
Papain, Inhibition by flour improvers (Elion).....	311
pH, see Hydrogen ion concentration	
Phosphated flour, see Flour, Phosphated	
Polarimetric method for starch determination (Earle and Milner).....	567
Potassium bromate, Efficiency as dough conditioner (Cathcart and Edelmann).....	575
Potato starch, see Starch, Potato	
Protamines, Effect on wheat mash fermentation (Balls and Harris).....	74
Proteases	
Inhibition by flour improvers (Elion).....	314
Soybean, activity (Laufer, Tauber, and Davis).....	267

**Protein**

- Corn, separation from starch in wet milling (Cox, MacMasters, and Hilbert)..... 447
- Fractions in zein (Gortner and MacDonald)..... 324
- Separation from starch in starch production (Dimler, Davis, Rist, and Hilbert)..... 430
- Wheat  
effect on farinograms, extensograms, and alveograms (Aitken, Fisher, and Anderson)..... 465
- effect of wheat-bug damage (Kretovich)..... 1
- errors in determination (Hildebrand and Koehn)..... 370
- 1943 western Canadian (Anderson, Neatby, and Levi)..... 283
- preharvest prediction correlation (Anderson, Neatby, and Levi)..... 283
- relation of Kjeldahl and Zeleny determination to loaf volume (Eva and Anderson)..... 560
- sulphydryl groups in (Myers and Working)..... 32
- Proteolytic enzymes**, see **Proteases**
- Rain**, Effect on wheat quality (Swanson) 126
- Reducing agents**, Effect on mixing time and bread quality (Swanson and Johnson)..... 222
- Reducing matter**, Effect of oxidizing agents on dough in presence of (Frellich and Frey)..... 241
- Riboflavin**  
Assays  
fluorometric method (Andrews)..... 398
- rapid fluorometric method (Hoffer, Alcock, and Geddes)..... 524
- sources of error in fluorometric method (Hoffer, Alcock, and Geddes)..... 515
- In flour and bread, fluorometric assay (Andrews)..... 398
- In rice varieties and milling products (Williams and Fieger)..... 540
- Rice**  
Nicotinic acid in varieties and milling products (Kik and Van Landingham)..... 154
- Riboflavin and biotin in varieties and milling products (Williams and Fieger)..... 540
- Rye meal**, Amylograph in testing (Brown and Harrel)..... 360
- Scab**, Effect on wheat quality (Merritt and Ansems)..... 199
- Self-rising flour**, see **Flour**, Self-rising
- Shear**, Resistance to in baked products, instrument for determining (Combs) 319
- Shortening tolerance**, Biscuit and self-rising flours (Schwain and Loving) 27
- Soda crackers**, see **Crackers**
- Sodium chloride**, Effect on dough (Swanson and Andrews)..... 140
- Sodium hypochlorite**, Germicidal action on thermophilic bacterial spores of wheat (Tremain)..... 511
- Soil fertility**, Effect on wheat quality (Worzella)..... 107

**Soybean**

- Amylases, see **Amylases**, Soybean
- Amylograph in testing products of (Brown and Harrel)..... 360
- Germinated, enzymes in (Lauter, Tauber, and Davis)..... 267
- Proteases, see **Proteases**, Soybean
- Spores**, Thermophilic bacterial, effect of chlorine compounds on in wheat (Tremain)..... 511
- Starch**  
Cereal, production from flours (Dimler, Davis, Rist, and Hilbert)..... 430
- Corn  
gelatinization (Anker and Geddes)..... 335
- improvement in determination (Earle and Milner)..... 567
- separation from protein in wet milling (Cox, MacMasters, and Hilbert)..... 447
- Polarimetric method for determination (Earle and Milner)..... 567
- Potato, gelatinization (Anker and Geddes)..... 335
- Production from  
stored and damaged wheat (MacMasters and Hilbert)..... 258
- wheat and other cereal flours (Dimler, Davis, Rist, and Hilbert)..... 430
- Wheat  
composition of amylopectin (MacMasters and Hilbert)..... 548
- fiber in amylopectin fraction (Stern) 369
- gelatinization (Anker and Geddes)..... 335
- improvement in determination (Earle and Milner)..... 567
- production from flour (Dimler, Davis, Rist, and Hilbert)..... 430
- production from stored and damaged wheat (MacMasters and Hilbert) 258
- Statistics**, Analysis and experimental design in (Goulden)..... 159
- Sulphydryl**  
Effect of  
added on dough (Swanson and Andrews)..... 140
- oxidizing agents in dough (Baker, Parker, and Mize)..... 97
- wheat germ fermentation on (Stern) 81
- In flour (Myers and Working)..... 32
- In wheat germ extracts, effect on dough (Stern)..... 81
- Sulfurous acid steep**, Effect in corn wet milling (Cox, MacMasters, and Hilbert)..... 447
- Thiamine**  
Assays  
simplification of the thiochrome method (Schiller)..... 544
- thiochrome method (Andrews)..... 388
- In breakfast cereals, loss on cooking (Lincoln, Hove, and Harrel)..... 274
- In flour, effect of wheat variations (Hoffer, Alcock, and Geddes)..... 210
- In flour and bread, thiochrome assay (Andrews)..... 388
- (Glick)..... 119
- In zwieback, loss during production (Meckel and Anderson)..... 280
- Thiochrome assay** for thiamine  
In flour and bread (Andrews)..... 388
- (Glick)..... 119



*Thiochrome assay (Continued):*

- Simplification (Schiller)..... 544  
**Toasting**, Thiamine loss in zwieback  
 (Meckel and Anderson)..... 280

**Viscosity**, Studies with the amylograph  
 (Brown and Harrel)..... 360

**Vitamin B<sub>1</sub>**, see Thiamine

**Vitamin B<sub>2</sub>**, see Riboflavin

**Wetting agents**

- Effect on  
 dough (Swanson and Andrews).... 140  
 mixing time and bread quality  
 (Swanson and Johnson)..... 222

**Wheat**

- Bug, damage to grain by (Kretovich) . 1  
 Canadian, relation of protein to loaf  
 volume (Eva and Anderson)..... 560  
 Damaged for starch production (Mac-  
 Masters and Hilbert)..... 258  
 Effect of  
 chlorine compounds on thermophilic  
 bacterial spores in (Tremain).... 511  
 frequent rains and storms on proper-  
 ties (Swanson)..... 126  
 growth environment on flour quality  
 (Sandstedt and Fortmann)..... 172  
 Flour, see Flour  
 Germ, effect of extracts on flour dough  
 (Stern)..... 81  
 Hard  
 effect of  
 growth environment on quality  
 (Sandstedt and Fortmann).... 172  
 scab on (Merritt and Ausemus).. 199  
 temperature on mixograms (Har-  
 ris, Sibbitt, and Scott)..... 374  
 variations on thiamine and ash  
 (Hoffer, Alcock, and Geddes).. 210  
 varieties on mixograms (Harris,  
 Sibbitt, and Elledge)..... 38

*Wheat (Continued):*

- Mash, fermentation, effect of prota-  
 mine (Balls and Harris)..... 74  
 Meal, mixograms (Lamb)..... 57  
 Protein content  
 effect on farinograms, extensograms,  
 and alveograms (Aitken, Fisher,  
 and Anderson)..... 465  
 errors in determination (Hildebrand  
 and Koehn)..... 370  
 1943 western Canadian (Anderson,  
 Neatby, and Levi)..... 283  
 preharvest prediction correlation  
 (Anderson, Neatby, and Levi)... 283  
 Quality  
 effect of  
 frequent rains and storms (Swan-  
 son)..... 126  
 scab (Merritt and Ausemus).... 199  
 soil fertility (Worzella)..... 107  
 evaluation with mixograms (Lamb) 57  
 — (Morris, Bode, and Heizer).... 49  
 Riboflavin in, rapid fluorometric  
 method (Hoffer, Alcock, and Geddes) 524  
 Soft  
 effect of varieties on  
 mixograms (Morris, Bode, and  
 Heizer)..... 49  
 sifted meal mixograms (Lamb)... 57  
 Starch, see Starch, Wheat  
 Stored, for starch production (Mac-  
 Masters and Hilbert)..... 258  
 Varieties, effect on malted wheat flour  
 quality (Meredith, Eva, and Ander-  
 son)..... 233  
 Wheat-bug damage to (Kretovich)... 1  
**Zein**, Fractionation (Gortner and Mac-  
 Donald)..... 324  
**Zeleny protein determination**, Relation  
 to Kjeldahl method and loaf volume  
 (Eva and Anderson)..... 560  
**Zwieback**, Thiamine loss during produc-  
 tion (Meckel and Anderson)..... 280



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